INTRODUCTION TO HISTOLOGY

Courses in Cell Biology usually precede those in Histology. In these courses Cell Concept or Cell Theory is studied and the cell is defined as the smallest basic structure of higher organisms capable of independent existence. Even today the Protozoa represent single-celled organisms. However, the higher organisms consist of tissues and organs. Tissues are groups of cells of similar function and origin (and also intercellular connective tissue in most cases) that form functional units. The organs represent an even greater measure of complexity and are composed of various tissues. At an even higher level of organization there are the organ systems composed of several organs (such as the gastrointestinal system, respiratory system, cardiovascular system, endocrine system). Thus the body can be seen to be formed of different levels of organization, with increasing levels of complexity and each of which plays important roles in the physiological homeostasis of the body.

LEVELS OF ORGANIZATION

Cells
Tissues
Organs
Organ Systems
Organism

The name "**Histology**" is derived from the Greek word for a tissue "*Histos*", and "*-logos*" = the study of. Today the concept of Histology as a subject includes far more than just the study of tissues, but includes understanding of the structure and function of cells, tissues, organs and organ systems, which can better be described as "**Microscopic Anatomy**". It is evident from the title that the study of Histology or Microscopic Anatomy involves the use of microscopes (light and electron) as basic tools. In addition to understanding the histology and ultrastructure of cells, tissues and organs, it is also necessary to complement the morphological observations with an understanding of the biochemistry, physiology and biophysics of these structures.

BASIC TISSUES

The body is composed of four basic tissues

(a) Epithelium
(b) Connective Tissue
(c) Muscle Tissue
(d) Nerve Tissue.

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 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 the 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 :

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

Metabolic functions of epithelium include :

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

(a) **tight junctions** (impermeable, enabling the organism to maintain the integrity of its internal environment)

(b) **adhering junctions** (**desmosomes**) promoting adhesion and reinforcing the structural integrity and sites for stress fibers

(c) **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 if the epithelial cells are described as :

(a) **squamous (**flattened)

(b) **cuboidal** (more square or cube-like)
(c) **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 (such as the columnar absorptive cells of the small intestine) are described as possessing an apical **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 (adenomas).

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:

(a) Exocrine glands, which secrete to the external environment via ducts.
(b) Endocrine glands, which secrete directly into the blood (and are also known as ductless glands)

(c) Mixed glands, which have both exocrine and endocrine secretions.

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:

(a) **unicellular glands**
(b) **multicellular glands**.

Unicellular exocrine glands

The main example of unicellular exocrine glands 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**.

(a) **Simple exocrine glands** have unbranched secretory ducts.
(b) **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.

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

(b) **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:

(a) **polypeptide (or protein)-secreting cells**
(b) **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).

CONNECTIVE TISSUE

Connective tissue is responsible for providing structural support for the tissues and organs of the body. This mechanical function 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:

(a) **cells**

(b) **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 :

(a) **protein fibers** (**collagen fibers**, **reticular fibers**, **elastic fibers**)

(b) amorphous ground substance

(c) **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

1. 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.

2. 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.

3. 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.

4. 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:

(a) **collagen fibers**
(b) **reticular fibers**
(c) **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:

(a) **glycine** (33.5%)
(b) **proline** (12%)
(c) **hydroxyproline** (10%)

The amino acids, **hydroxyproline** and **hydroxylysine** are characteristic of collagen. It 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 - 2μm) 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 stained with 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 networks around cells and cell groups in diverse organs. They are abundant in lymphatic organs (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 (20-30μm diameter) in connective tissue characterized by cytoplasm packed with large round **basophilic granules** (up to 2μm 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:

1. **Loose Connective Tissue**
2. **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:

(c) **dense irregular connective tissue**
(d) **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.

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 epipiphyses 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:

(a) Hyaline cartilage
(b) Elastic cartilage
(c) Fibrocartilage

The differences 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:

(a) appositional growth
(b) 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 the 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 shows basophilia 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:

(a) intervertebral disks
(b) pubic symphysis
(c) temporo-mandibular joints
(d) at sites of connection of many ligaments to bones
(e.g. *Ligamentum teres femoris*)
(e) 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:

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

FUNCTIONS OF CARTILAGE

Cartilage is important for:

(a) **skeletal support** in the embryo prior to the development of the bony skeleton.
(b) **elongation of developing long bones** (endochondral ossification).
(c) **articulating joints** (articular cartilage).
(d) **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

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

(a) 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.
(b) Bone provides **protection for the vital organs** of the body: the skull protects the brain; the ribs protect the heart and lungs.
(c) The **hematopoietic bone marrow** is protected by the surrounding bony tissue.
(d) 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:

(a) mechanical stimuli
(b) metabolic causes (lack of dietary calcium, illness, aging)
(c) endocrine changes
(d) effects of drugs.

MACROSCOPIC STRUCTURE OF BONE

There are two main categories of bone :

(a) **Spongy bone** (trabecular bone, cancellous bone)
(b) **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:

(a) **long bones** (e.g. humerus, femur)
(b) **flat bones** (membrane bones)
(c) **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.

(a) **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).

(b) **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).

(c) **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 :

(a) **Organic matrix**
(b) **Inorganic salts**.

Organic matrix

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

(a) **sulfated glycosaminoglycans** :

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

(b) 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:

(a) Osteoprogenitor cells
(b) Osteoblasts
(c) **Osteocytes**
(d) 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 (20-100μm diameter) and are multinuclear (with up to 50 nuclei). Osteoclasts are involved in **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**". The cells are metabolically very active, possess large numbers of mitochondria (resulting in the acidophilia of regular staining) and have well-developed Golgi bodies. Osteocytes synthesize and 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** :

(a) **Osteons** (**Haversian Systems**)
(b) **Circumferential Systems**
(c) **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 20-110μm. 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):

1. **Intramembranous ossification**
2. **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.

1. The first stages involve the development of a **hyaline cartilage model** with surrounding **perichondrium**.

2. A layer of woven bone (the **periosteal collar**) develops around the central shaft of the cartilage as a result of **intramembranous ossification**.

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

   The chondrocytes in the developing central shaft (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 the matrix 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 mesenchyme cells and begin forming primary bone tissue on the calcified cartilage framework.

4. 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.

5. 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:

(a) **resting zone** (small flattened lacunae)
(b) **zone of proliferation** (site of mitoses, and larger elliptical lacunae)
(c) **zone of hypertrophy** (greatly enlarged and rounded chrondrocytes in enlarged lacunae)
(d) **zone of calcification** of the matrix and degeneration of the chondrocytes
(e) **zone of ossification**. Osteoblasts are involved in forming bone trabeculae on the remains of the calcified cartilage.
(f) **primary spongiosa**, 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.

6. **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.

7. **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**:

1. **Parathyroid hormone**
2. **Calcitonin**
3. **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**.

MUSCLE TISSUE

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

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

(a) **Skeletal Muscle**
(b) **Cardiac Muscle**
(c) **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 connective tissue 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 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 **α-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

1. Each myofiber is innervated by efferent nerve impulses from axon terminals of motor end plates.

2. The nerve impulse causes depolarization of the sarcolemma and this depolarization continues in the T-tubule.
3. On reaching the triad the impulse causes the release of accumulated calcium ions from the terminal sacs of the sarcoplasmic reticulum into the sarcoplasm.

4. 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.

5. The process is energy dependent involving mitochondrial ATP and ATP-ase activity from the heavy meromysoin.

6. 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.

7. 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

1. **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 (β-particles) are seen individually or in small clusters).

2. 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.

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

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

5. 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:

1. **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.

2. **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.

3. **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 the Type I fibers. White muscle ("white meat") is dominated by the 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 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 have typical histological appearance of striated muscle, however they 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:

1. The muscle **fibers branch** (bifurcate) and are arranged in series to form an anastomosing network.
2. Each myocyte has one or two **central nuclei** (unlike the many peripheral nuclei of syncytia of skeletal muscle fibers).
3. The fibers have more sarcoplasm.
4. The mitochondria are larger and better developed.
5. **All the fibers are Type I** (red fibers, with abundant myoglobin, high oxidative slow-twitch).
6. **Glycogen** is more common.
7. The myocytes have specialized areas of contact - the **intercalated disks**.
8. **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:

1. **transverse regions**, rich in desmosomes and tight junctions. These are important in providing good cell adhesion between adjacent myocytes.
2. **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.

(a) **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.

(b) **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.

Unlike skeletal muscle fibers, cardiac muscle fibers lack motor end plates.

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**.

Unlike striated and cardiac muscle, smooth muscle lacks cross-striations. 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

1. 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.
2. Smooth muscle is found in the **walls of the respiratory tracts**.
3. Smooth muscle is present in the **walls of blood vessels** (vascular smooth muscle, especially in arterial vessels).
4. Smooth muscle is found in the **dermis of the skin** (arrector pili).

5. Smooth muscle is found in the eye (**iris diaphragm**) controlling the amount of light reaching the retina.
6. Smooth muscle is important in the **uterus**.
   In addition, smooth muscle is 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 20μm. In most cases the thickness of the fibers at their widest part as seen in cross section is typically about 5-10μm.

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:

(a) **thin myofilaments** (5-7nm thick), which are the most common type
(b) **thick myofilaments** (about 16nm thick), which are less common
(c) **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 mechanism of contraction 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).

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:

(1) **Central Nervous System** (CNS), consisting of the brain and spinal cord.
(2) **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:

(1) **Nerve cells** (Neurons)
(2) **Neuroglia**.

Central Nervous System (CNS)

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, and a complicated network of cell processes (**neuropil**).

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.

In the Peripheral Nervous System (PNS), perikarya 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:

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

The morphofunctional unit of the nervous system is the neuron. 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:

(1) **Dendrites**
(2) **Perikarya** (cell bodies)
(3) **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 150μm), whereas others are very small (perikarya of only 4-5μm).

Morphological classification of neurons

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

(1) **Unipolar** (**pseudounipolar**) **neurons** have a single process (axon). These are found in sensory ganglia of dorsal roots of spinal nerves.

(2) **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).

(3) **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.

(1) **Sensory neurons**. These receive sensory stimuli from the environment (from receptors) and from within the body (e.g. unipolar neurons).

(2) **Motor neurons**. These control the effector organs (muscles, exocrine glands, endocrine glands)

(3) **Interneurons** (Intermediate neurons). These are typically found in the CNS and connect between other neurons (often between sensory and motor neurons).

(4) **Neurosecretory neurons**. These are specialized neurons that synthesize and secrete hormones.

Each neuron has 3 physiological parts or segments:

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

(2) **Conductive segment** (axon)

(3) **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.

1. Stimulation of the **receptor** (in skin or skeletal muscle spindle)
2. Propagation of an impulse via **afferent sensory nerve** (unipolar neuron), which enters the gray matter of the spinal cord.
3. **Interneurons** connect with cell body of motor neuron in ventral horn.
4. **Motor neuron** transmits the efferent impulse to an effector.
5. 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:

(a) sites of axon collaterals
(b) large concentrations of mitochondria in the axon at these sites (high local metabolic activity)
(c) site of saltatory conduction (non-decremental)
(d) 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:

(1) **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 regeneration of the axon.

(2) **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.

(1) **Astrocytes** (or Astroglia)
(2) **Oligodendrocytes** (or Oligodendroglia)
(3) **Microglia**
(4) **Ependymal cells**

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

Neuroglia differ from neurons:

(1) Neuroglia have **no action potentials** and cannot transmit nerve impulses
(2) Neuroglia **are able to divide** (and are the source of tumors of the nervous system)
(3) Neuroglia **do not form synapses**
(4) 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:

(a) **Protoplasmic astrocytes**. These are present in the gray matter of the brain and spinal cord. Their processes are relatively thick.

(b) **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

(1) **Structural support** (especially the astrocytes in the CNS)

(2) Participation in the **blood-brain-barrier** (astrocytes)

(3) **Formation of the myelin sheath** of axons (oligodendrocytes)

(4) **Isolation of junctional surfaces of synapses**

(5) **Repair processes** following damage or injury to nerves.

NERVE STAINING TECHNIQUES

1. **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).

2. **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.

3. **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:

(a) **axo-dendritic**
(b) **axo-somatic**
(c) **dendro-dendritic**
(d) **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.

If synapses are examined by transmission electron microscopy the terminal bulb is seen to contain membrane-bound **synaptic vesicles** (25-65μm), which store the **neurotransmitter**. 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 or **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:

(a) Spinal ganglia (**Dorsal root ganglia**)
(b) 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:

(a) chromatolysis (loss of Nissl bodies) with the remaining Nissl moving to the periphery of the perikaryon
(b) swelling of the perikaryon
(c) 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.

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:

(1) **Heart** (muscular pump)
(2) **Pulmonary circulation** (system of blood vessels to and from the lungs)
(3) **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:

(1) **Conducting** or **Elastic Arteries**.
These are large arteries closest to the heart (aorta, renal artery) with very high blood flow (320mm/sec in the aorta).

(2) **Distributing** or **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**.

In the Systemic Circulation the arterial blood 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 the 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 an enormous surface area (700-1000m^2) and in total weigh about 1.5kg.

Blood capillaries

Blood capillaries have a diameter of about 7-9μm, which is close to the dimensions of erythrocytes (about 7.2μm). 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) :

(1) **Continuous capillaries**
(2) **Fenestrated capillaries**
(3) **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 :

(1) **micropinocytotic vesicles** in the endothelium (as in continuous capillaries)
(2) **fenestrations** (as in fenestrated endothelium or sinusoids)

or **intercellular** via:

(3) **gap junctions**
(4) **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 muscle cells and may even be phagocytic.

Endothelial cells are known to produce a variety of local factors that are important in the functioning of the cardiovascular system. 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.

1. ***Tunica intima***

This is the most internal tunic and consists of:

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

2. ***Tunica media***

This middle tunic consists of:

(a) circular or spiral bands of orderly arranged **smooth muscle**.
(b) concentric elastic lamina (formed by the smooth muscle cells).

3. ***Adventitia***

This outer layer is composed of:

(a) connective tissue surrounding the vessel.
(b) outer elastic limiting membrane (on the border between the *Tunica media* and the *Adventitia*.
(c) ***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:

(a) ***Tunica intima*** with endothelium alone (no subendothelial layer) and a very thin inner elastic limiting membrane
(b) ***Tunica media*** with only 4-5 layers of smooth muscle
(c) ***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 due to occlusion of the lumen of arteries are two of the 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:

(1) more numerous than arteries
(2) diameter of vessels is larger than that of adjacent arteries
(3) 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)
(4) the relative numbers of *vasa vasorum* are greater in the veins (necessary as the vessels have much less oxygenated blood)
(5) 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 (20-50μm). 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

(1) 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.
(2) The lymph vascular system plays a key role in homeostasis of the volume of extracellular fluid.
(3) The lymph vascular system also returns lymphocytes from the lymph nodes to the blood.
(4) The system also transports immunoglobulins (antibodies) from the lymph nodes 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.

(1) Typical lymph capillaries have a diameter of 10-50μm only. Lymph vessels have very thin walls. The wall of the lymph capillary is composed of a single layer of endothelium (about 0.3μm thick). Lymph vessels are hard to detect in histological preparations as the lumen tends to collapse.
(2) Lymph capillaries (unlike endothelial cells of blood vessels) lack a basal lamina.
(3) No pericytes or adventitial cells are found.
(4) They lack marginal folds (as seen in ultrastructure of blood capillaries).
(5) 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.

PERIPHERAL BLOOD

The blood circulatory system is unidirectional. Adult males have about 5.5 liters blood (slightly less in females).

Blood consists of:

(a) **Formed elements** (cells and platelets)
(b) **Plasma** (liquid component in which the formed elements are suspended).

Plasma is an aqueous solution containing proteins (7%), lipoproteins (10%), amino-acids, vitamins, hormones, and inorganic salts. The main blood proteins are:

(a) albumins (maintains osmotic pressure of blood)
(b) gamma globulins (**immunoglobulins** or **antibodies**)
(c) fibrinogen (clotting agent)

If blood is allowed to clot, the clot contains the formed elements, whereas the clear yellow liquid that separates is known as the **serum**. Serum is similar to plasma but lacks fibrinogen and other clotting agents.

If a test-tube of blood is centrifuged it separates into several distinct layers. The lowest layer, which is an orange or reddish color, contains the **erythrocytes** (about 45% of the volume). A thin layer (1%) known as the **buffy coat** is located above the layer of erythrocytes. This contains the **leukocytes** and **platelets**. The remaining upper clear fluid is the **plasma** (about 55%). The relative volumes of cells and plasma are described as the "**hematocrit**". Low hematocrit values can indicate blood disorders such as anemia.

Blood smears

A drop of blood spread thinly on a microscope slide and dried is known as **a blood smear**. In order to provide contrast and distinguish the various cell types (formed elements) the smear is stained. The various common blood staining techniques (Leishman, Giemsa, Wright) are all modifications of the Romanovsky (1891) staining method based on mixtures of methylene blue (basic dye) and eosin (acid dye). The methylene blue is oxidized by certain components to form **azures** (purple color), and the structures stained are described as azurophilic. Affinity for eosin is termed eosinophilia or acidophilia.

Formed elements of the blood

(1) **Erythrocytes** (red blood cells)
(2) **Leukocytes** (white blood cells)
(3) Platelets

Erythrocytes

The erythrocytes of mammals lack nuclei (apart from in the fetus). Human erythrocytes are biconcave discs about 7.2μm diameter (similar to the dimensions of most capillaries). Abnormal variation is size is known as **anisocytosis**. Abnormal variation in shape is known as **poikilocytosis**. Erythrocytes are fairly flexible and can modify their shape to pass bends in the capillaries. Erythrocytes transported in the blood often appear bound together in "***rouleaux***", similar to stacks of coins.

The concentration of erythrocytes in adult males is about 5.5 million/μL and slightly less in females.

Erythrocytes are acidophilic and are stained a reddish color with eosin. The main function of the erythrocytes is the binding and transport of oxygen to the tissues. The main protein of erythrocytes is **hemoglobin**, to which oxygen is bound to form **oxyhemoglobin**. Erythrocytes circulate in the blood for about 120 days prior to removal of the aged erythrocytes and their destruction by macrophages mainly in the spleen and liver.

Erythrocytes are sensitive to osmotic pressure. In **hypertonic solutions** the erythrocytes shrink and become crenated, whereas in **hypotonic solutions** they swell and burst (**hemolysis**) leaving membrane "ghosts".

Leukocytes (white blood cells)

The leukocytes are involved in the defense of the body and participate in protection against foreign material including microorganisms. The leukocytes are classified into two major groups:

(a) Granulocytes (**Neutrophils**, **Basophils**, **Eosinophils**)
(b) Agranulocytes (**Lymphocytes**, **Monocytes**)

Granulocytes are characterized by:

(i) irregular segmented nuclei
(ii) specific granules (specific size, staining affinities, ultrastructure)
(iii) terminal (fully differentiated) cells.

Agranulocytes are characterized by:

(i) regular nuclei (round or kidney-shaped)
(ii) non-specific granules (also present in other leukocytes)

Leukocytes can leave the blood circulatory system and enter connective tissue, where they mostly function. This ability to cross the capillary walls is known as **diapedesis**. This migration of leukocytes from capillaries into the connective tissue is important in tissue responses to injury. The leukocytes do not return to the blood, with the exception of lymphocytes (recirculation).

GRANULOCYTES

Neutrophils

Neutrophils, also known as **polymorphonuclear leukocytes** or **polymorphs**, are the most common of the leukocytes and represent some 55-70% of all the circulating leukocytes. They have a diameter of about 12μm and are characterized by a segmented nucleus. In females a small "**drumstick**" is found (**Barr's body**) on the nucleus representing a condensation of the sex-chromatin. Immature (**juvenile**) forms of neutrophils (about 1-2%) have a non-segmented rod-like or horseshoe-shaped nucleus ("**band**").

The cytoplasm of neutrophils is packed with **small specific granules** (up to 200 per cell) stained a salmon pink color in smears after Romanovsky-type staining. Some specific granules contain **phagocytins**, which are anti-bacterial substances involved intracellular digestion. The **non-specific granules** (stained weakly azurophilic) contain lysosomal enzymes and peroxidase.
Neutrophils are phagocytic cells involved as a first line of defence against invading microorganisms. They are important in inflammation and at sites of injury or wounds. **Pus** that develops in sites of infection is mainly composed of dead neutrophils.

Eosinophils

Eosinophils represent 1-4% of leukocytes. They typically have **bilobed nuclei** and fairly large (0.5-1.5μm) **acidophilic granules**. The color of the granules in stained smears is similar to that of erythrocytes. The granules are considered to be **lysosomes** and contain acid phosphatase, cathepsin and other lysosomal enzymes. At the ultrastructural level the granules are seen to possess a characteristic central **crystalloid** (internum). Eosinophils are involved in

selective phagocytosis. An increase in the numbers of eosinophils is seen in allergic reactions or helminth parasitic infections.

Basophils

These represent only about 1% of the leukocytes. Their nucleus is bilobed or S-shaped. They have very large, **irregular basophilic granules**, that commonly obscure the nucleus. The granules (similar to those of mast cells) contain **histamine** and **heparin** and show metachromatic staining. In stained blood smears the granules stain a violet color. Basophils release the contents of their granules in response to antigens.

AGRANULOCYTES

Lymphocytes

Lymphocytes represent 25-30% of all the leukocytes. Lymphocytes are involved in the immune responses of the body. The most common lymphocytes are small cells (6-8μm diameter), with very dense regular nuclei, with relatively little cytoplasm. The different types of lymphocytes are considered in the framework of the lymphatic system.

Monocytes

Monocytes represent about 5% of the leukocytes. They possess oval or kidney-shaped nuclei, often eccentrically located. The nuclei are less intensely stained than those of lymphocytes. The cytoplasm is basophilic with small azurophilic granules (lysosomes). Monocytes are part of the **Mononuclear Phagocyte System**. They are non-terminal cells and can differentiate into phagocytic cells such as macrophages. Monocytes that enter the tissues do not return to the blood.

Blood platelets

Blood platelets are derived from the cytoplasm of **megakaryocytes** of the bone marrow. They are small (2-5μm) with a central core of small purple granules (**granulomere**) surrounded by an outer pale-blue **hyalomere**. At the periphery (as seen by electron microscopy) there are peripheral or marginal microtubules. If a blood vessel is injured, the platelets are involved in the clotting reaction. They aggregate at the site of injury, change shape and help form the thrombus.

THE DIGESTIVE SYSTEM

The digestive system consists of the **digestive tract and associated glands**.

The main components of the digestive tract are the:

(a) Oral cavity
(b) Esophagus
(c) Stomach
(d) Small Intestine
(e) Large Intestine
(f) Rectum and anus.

The entire digestive tract can be considered as a hollow tube surrounded by a wall composed of four main layers:

1. ***Mucosa***
2. ***Submucosa***
3. ***Muscularis***
4. ***Serosa*** *or* ***Adventitia***

1. ***Mucosa***

The mucosa consists of :

(a) **epithelium** (lining the lumen)
(b) ***lamina propria*** (loose connective tissue)
(c) ***muscularis mucosae*** (thin layer of smooth muscle cells).

The **epithelial lining of the mucosa** forms a selective barrier between the external environment (lumen) and the body. All the food products that are digested and absorbed by the body need to pass through the epithelial lining. This epithelial lining may contain **goblet cells**, that secrete mucus for lubrication. **Endocrine cells** (part of the diffuse endocrine system) are common in the epithelium and produce polypeptide hormones, that play a role in the regulation of the digestive processes.

The ***lamina propria***, situated just below the epithelium, consists of loose connective tissue, with an abundant blood supply. Lymphatic nodules, lymphocytes and plasma cells, and macrophages are common in the lamina propria and form a first line of immunological defense against bacterial and viral invasion.

The ***muscularis mucosa*** causes local muscular contractions in the mucosa.

2. ***Submucosa***

The ***submucosa*** consists of **dense connective tissue** and **Meissner's nerve plexus**.

3. ***Muscularis***

The **muscularis** consists of two sub-layers of **smooth muscle cells** (typically **inner circular** layer, **outer longitudinal** layer). These are involved in the peristaltic movements of the intestine. Between these two muscle layers is **Auerbach's (myenteric) nerve plexus**. The rhythmic peristaltic contractions of the muscularis are responsible for propelling and mixing food in the digestive tract. These movements are mainly generated by Auerbach's myenteric plexus. The neurons of the plexus can be visualized by silver impregnation techniques. It can be shown that the plexuses consist of aggregates of nerve cells in the form of small parasympathetic ganglia.

4. ***Serosa*** or ***Adventitia***

The **serosa** consists of a thin layer of loose connective tissue covered by a **simple squamous epithelium** (mesothelium). **Serosa** is present in the parts of the intestinal tract that are present in the peritoneal cavity. The regions of the intestinal tract that are not present in the peritoneal cavity are held in place by an outer layer of loose connective tissue (***adventitia***).

ESOPHAGUS

The esophagus is a straight muscular tube connecting the oral cavity to the stomach. The esophagus contains the four basic layers common to the rest of the digestive tract.

The esophagus is lined with **stratified squamous epithelium** (without keratin).

The lamina propria near the stomach contains mucus-secreting **esophageal cardiac glands**.

Mucus-secreting **esophageal glands are present in the submucosa**. (This is the only site in the intestinal tract, apart from the duodenum, where exocrine glands are present in the submucosa.)

The ***muscularis*** in the upper third of the esophagus is composed of striated muscle cells (non-voluntary muscle). In the mid-region of the esophagus the *muscularis* has a mixture of striated muscle and smooth muscle. The muscles of the lower third of the esophagus are only smooth muscle.

The outermost layer of the esophagus consists of adventitia, apart from a small portion of the esophagus that extends into the peritoneal cavity (serosa).

STOMACH

The stomach is a very muscular organ in which acid secretions and digestive enzymes contribute to the digestion of food. From a histological viewpoint the stomach can be divided into two major histological regions:

(a) Fundus and **Body**
(b) Pylorus

FUNDUS AND BODY

These are lined with a **homogeneous simple columnar epithelium** consisting of mucus-secreting cells. Mucus secreted by these cells provides protection from the highly acidic contents of the lumen. The surface epithelium invaginates into the lamina propria to form **gastric pits**. The **gastric glands of the fundus and body** are **branched tubular glands**, which open into the gastric pits. These tubular glands consist of:

(a) **Stem cells** (in the neck region). These cells proliferate rapidly and migrate to replace the stomach epithelial lining cells (whose turnover time is short, about 4 days, owing to the harsh acidic environment). These stem cells also migrate deeper into the glands and differentiate into the various secretory cells of the glands.

(b) **Mucous neck cells**. These cells, also present in the neck region, secrete mucus (stained intensely with PAS), with staining characteristics that differ from the surface epithelial lining cells.

(c) **Parietal cells** (oxynitic cells). These are mainly found in the upper part of the gastric glands. These are rounded or pyramidal cells characterized by their **round central nuclei** and very **acidophilic cytoplasm** (owing to the large number of mitochondria). These cells possess **intracellular canaliculi** with microvilli opening to the lumen (difficult to see in wax-sections). The parietal cells are responsible for the synthesis and secretion of hydrochloric

acid. In humans these cells are also responsible for synthesis of **intrinsic factor**, a glycoprotein essential for intestinal vitamin B_{12} absorption.

(d) **Chief cells** (**Zymogen cells**). These are present mainly in the deeper parts of the glands. These cells are involved in synthesis and secretion of digestive enzymes and consequently display the features of protein synthesizing and exporting cells. This is seen as **basophilic staining** in the more basal regions of the cells (areas of rough endoplasmic reticulum). The **zymogen granules** that accumulate in the apical regions of the cells, contain the proenzyme **pepsinogen**, which is converted into the proteolytic enzyme, **pepsin**, when secreted into the acid environment of the stomach.

(e) **Enteroendocrine cells**.

PYLORUS

The **gastric pits** of the pylorus are longer and wider than the pits of the fundus/body, however the gastric glands are shorter and more coiled. These glands consist almost entirely of **mucus-secreting cells** (there are no parietal or zymogen cells). The gastric gland cells also secrete **lysozyme**.

The endocrine cells of the pylorus include cells secreting **gastrin** (which stimulates acid secretion by the parietal cells).

The ***muscularis*** of the stomach is composed of three layers of smooth muscle: an **external longitudinal layer**, a **middle circular layer**, and an **internal oblique layer**. These layers are not always easily distinguishable in histological sections. The **pyloric sphincter**, which controls the discharge of stomach contents to the duodenum, consists of an enlarged middle layer of smooth muscle.

SMALL INTESTINE

The small intestine is the main site of absorption of digested food. The small intestine is specialized for the completion of the digestion processes and the subsequent absorption of the digested products. The overall length of the small intestine is about 5 meters, and consists of three main segments:

(a) Duodenum
(b) Jejunum
(c) Ileum

Characteristic features of the small intestine include:

(a) **Intestinal villi**. These are finger-like projections into the lumen (consisting of surface epithelium and underlying lamina propria).

The **epithelium** lining the lumen consists of a **simple columnar heterogeneous epithelium with goblet cells**. The apical surface of the absorptive epithelial cells has a "**brush border**" (resulting from an orderly arrangement of closely-packed microvilli, which may number several hundred per absorptive cell). The microvilli, as seen by transmission electron microscopy, have a central core of actin filaments. The main function of the microvilli is to increase the surface area available for absorption. The absorptive cells have oval nuclei, typically in the basal half of the cells.

The ***lamina propria*** of the small intestine is formed from loose connective tissue. This contains blood vessels, nerves, large lymphatic vessels (site of absorption of lipids), and cells of the immune system, often in the form of lymphatic nodules.

(b) **Intestinal glands**. These are simple tubular glands that open to the intestinal lumen between the base of the villi. The intestinal glands are sometimes called the **crypts of Lieberkuhn**. Secretory cells (**Paneth cells**) with large acidophilic granules are found at the base of the intestinal glands. Their function is still not fully understood, but it is known that they secrete **lysozyme**, which has anti-bacterial properties.

(c) **Valves of Kerckring**. The lining of the small intestine has permanent folds known as Valves of Kerckring or ***Plicae circulares***. These are most prominent in the jejunum. These folds, seen macroscopically in transverse sections, consist of mucosa and submucosa.

DUODENUM

The main distinguishing feature of the duodenum is the presence of **glands in the submucosa**. These duodenal or **Brunner's glands** produce alkaline secretions to counteract the effects of gastric acids that reach the duodenum. They also provide the necessary alkaline environment for the functioning of the exocrine pancreatic secretions.

JEJUNUM

The main distinguishing feature of the jejunum is the presence of prominent **Valves of Kerckring *(plicae circulares)***.

ILEUM

The ileum is almost devoid of Valves of Kerckring, however **large accumulations of lymphatic tissue**, both nodular and dense, are found in the *lamina propria*. These can often be seen macroscopically as large white patches and are commonly known as **Peyer's Patches**.

LARGE INTESTINE

The large intestine lacks folds or villi. It is characterized by many tubular **intestinal glands with large numbers of goblet cells**. This is sometimes described as a **glandular epithelium**.

The large intestine is the **site of water absorption** (via columnar absorptive cells) and is also the site of **formation of the feces**. The secretions of the goblet cells provide lubrication for the luminal surfaces. Abundant lymphatic tissue is common in the *lamina propria* (owing to the large bacterial population in the lumen of the large intestine).

Whereas the circular smooth muscle layer is continuous, the longitudinal smooth muscle of the muscularis is in the form of three thick bands, known as **teniae coli**.

The **anal region**, unlike the rest of the large intestine, has a series of **longitudinal folds** and the epithelium becomes a **stratified squamous epithelium**.

APPENDIX

The appendix is a blind-ended tube with the basic histological structure of the large intestine. The lumen is usually fairly narrow and irregular. Lymphatic tissue (nodular and dense) is abundant in the wall of the appendix.

THE LIVER

The liver is located in the peritoneal cavity below the diaphragm. The liver is the largest internal organ of the body and can reach 1,500 g in adults. It is also the largest gland of the body and serves both exocrine and endocrine functions. The metabolic functions of the liver are numerous.

The liver has an abundant blood supply and in particular receives venous blood from the small intestine (mesenteric veins and hepatic portal vein), which contains all the food components absorbed from the intestine apart from the lipids.

Histological organization of the liver

The liver has four **lobes**. The basic morphofunctional units are **hepatic lobules**, which number approximately one million. In some animals, such as the pig, the lobules have a hexagonal appearance as seen in sections. The diameter of each lobule is about 1-2mm. A thin layer of connective tissue surrounds each lobule. Overall there is very little connective tissue in the liver. At the center of each lobule is a **central vein**. Each lobule has a vascular supply of arterial blood (from branches of the hepatic portal artery) and venous blood (from branches of the hepatic portal vein) from specific areas at the angles of the hexagon. These areas are known as **portal areas** as they receive arterial and venous blood from the porta of the liver.

Portal areas

The structures found in each portal area are:

(a) **branch of the hepatic portal vein** (the largest vessel in the portal area, bringing about 70% of the afferent blood)
(b) **branch of the hepatic artery** (arteriole supplying about 30% of the afferent blood)
(c) **bile ductule** (epithelial structure, through which bile produced by the liver cells is secreted)
(d) **lymph vessels** (the liver is the source of 35-50% of all lymph in the body under resting conditions). These lymph vessels are sometimes difficult to detect in standard histological preparations owing to the thinness of the walls and collapse of the lumen.

The structures of the portal area are surrounded by loose connective tissue (part of Glisson's capsule). In cases where the borders of the lobules are difficult to detect, the portal area provides a useful landmark or reference point.

The arterial and venous blood flows centripetally in each lobule (from the portal areas to drain in the central vein), whereas the bile flows centrifugally towards the portal areas.

The main cells of the lobules are epithelial cells known as the **hepatocytes**. These are sometimes referred to as **parenchymal cells**. The hepatocytes are derived from the embryonic endoderm. The hepatocytes in each lobule are arranged in radial **hepatic plates**. The blood flows between the hepatic plates in large **sinusoids** and as a result all the hepatocytes have a rich blood supply.

Hepatic sinusoids

The sinusoids are discontinuous and fenestrated.

The four main cell types of the sinusoids are:

1. **Endothelial cells**
2. **Kupffer cells**
3. **Fat-storing (Ito) cells**
4. **Pit cells (NK cells)**.

(1) **Endothelial cells**

The endothelial cells lack any basal lamina and their structural support comes from reticular fibers in the space separating the endothelial layer from the hepatocytes. This space is known as the **Space of Disse**. The Space of Disse is visible in histological preparations as a gap between the free edge of hepatocytes and the endothelial cells.

(2) **Kupffer cells**

The Kupffer cells are **fixed macrophages**, which belong to the **Mononuclear Phagocyte System**. They are associated with the endothelial cells and possess cellular processes, which extend via the spaces between endothelial cells into the lumen of the sinusoid. The location and phagocytic function of Kupffer cells can be easily demonstrated if a vital dye, such as Trypan blue, is injected into the peritoneal cavity or into a blood vessel; within a short time, the dye is engulfed into the cytoplasm of Kupffer cells. Kupffer cells also take part in the breakdown of aged erythrocytes. In addition, the Kupffer cells can detect and engulf bacteria in the blood and lead to their breakdown.

(3) **Fat-storing cells**

These are also known as **lipocytes** or **Ito** cells. They are typically found in the Space of Disse and have the ability to accumulate lipid droplets. They are the main source of vitamin A storage in the body and also play a role in wound healing (hepatic fibrogenesis).

(4) **Pit cells**

These are mobile cells found in the Space of Disse that belong to the immune system. They are believed to be a form of Natural Killer cells (NK cells) or Lymphokine-activated Killer cells (LAK cells).

Histology of hepatocytes

Hepatocytes are large (20-30μm) polyhedral epithelial cells with large central regular nuclei. About 25% of all the hepatocytes have two nuclei (**binucleate**), and there are several instances (up to 10%) of **polyploid hepatocytes** with very large nuclei (more than one set of chromosomes).

The cytoplasm of the hepatocytes is typically fairly acidophilic (many mitochondria present), with areas of basophilia (rough endoplasmic reticulum). The hepatocytes are unusual in that they possess abundant **rough endoplasmic reticulum** (RER) and **smooth endoplasmic reticulum** (SER) in the same cells. The RER is associated with protein synthesis, whereas the SER is associated with steroid metabolism.

Golgi bodies of the hepatocytes are well developed

Lipid droplets are commonly found in the cytoplasm. These increase in number and size after ingesting alcohol and also after treatment with many steroid drugs such as glucocorticoids.

Lysosomes are common in hepatocytes, especially in the vicinity of the bile canaliculi and are sometimes referred to as **peribiliary bodies**.

Peroxisomes (formerly called microbodies) are also common in hepatocytes. These are membrane-bound organelles, typically 0.2-0.8μm in diameter, which can be identified at the ultrastructural level by the presence of crystalline nucleoids. The peroxisomes contain enzymes such as catalase, urate oxidase, d-amino oxidase and gamma-hydroxy acid oxidase. The peroxisomes play a role in protecting the cells from effects of peroxides, which may cause irreversible damage.

Glycogen deposits in the cytoplasm can be shown with periodic acid-Schiff staining. At the ultrastructural level the glycogen is seen to be in the form of rosettes.

Bile canaliculi

The membranes between adjacent hepatocytes are modified to form small channels, known as **bile canaliculi**, through which the bile secreted by the hepatocytes initially passes. The bile canaliculi possess **microvilli** and are sealed by **tight junctions** to prevent bile leakage. The bile canaliculi form a complex anastomosing network, which in the portal areas open into **Hering** canals, lined with cuboidal epithelium, and into the portal bile ductules. The network of bile canaliculi in hepatic lobules can be demonstrated in histochemical preparations of liver processed for ATP-ase enzymatic activities.

FUNCTIONS OF HEPATOCYTES

(a) Hematopoietic function

In embryos the liver functions as an haemopoietic organ and is active in producing blood cells (erythrocytes and leukocytes). At week 10 the liver is about 10% of fetal weight. The hematopoietic function lasts until month 7 of fetal development. Histological examination of the fetal liver shows large nests of forming blood cells between the hepatocytes and the walls of blood vessels.

(b) Metabolic functions of the liver

1. **Deamination of amino acids**. The liver is responsible for the breakdown of amino-acids. The hepatocytes have all the necessary enzymes of the urea cycle. The urea is transported to the kidneys prior to excretion in the urine.

2. **Synthesis of plasma proteins**. The hepatocytes are the main source of important plasma proteins such as: **albumin**, **globulin**, **fibrinogen**, **prothrombin**. Unlike other protein synthesizing and secreting cells of the body, the hepatocytes are unusual in that they lack protein storage granules.

3. **Carbohydrate metabolism**. The hepatocytes play major roles in glucose homeostasis. Excess carbohydrates can be stored as glycogen deposits, which can be reconverted to glucose according to need (**glycogenesis** and **glycogenolysis**). The hepatocytes are also the main site of **gluconeogenesis** (formation of glucose from amino-acids or lipids) in times of stress such as starvation.

4. **Lipid metabolism**. The hepatocytes are the site synthesis and secretion of **serum lipoproteins**. They also play major roles in **cholesterol and steroid metabolism** and in the **metabolism of the fat-soluble vitamins** (vitamins A and D**).**

5. **Drug detoxification**. The hepatocytes are important in the detoxification of lipid-based drugs. The SER is responsible for the "conjugation" process involved in the inactivation of the drugs.

6. **Source of lymph**. The liver produces large quantities of lymph (50% of protein-rich lymph).

7. **Bile synthesis**. The hepatocytes synthesize bile, which plays a role in the emulsification of fats prior to lipid absorption into the lacteals of the villi of the small intestine. Pigments resulting from the breakdown of aged erythrocytes, such as bilirubin, are excreted in the bile.

Synthesis of serum lipoproteins

Up to 50% of the protein-rich lymph is derived from the liver. Fatty acids from the blood are taken up by the hepatocytes and become esterified in the smooth endoplasmic reticulum (SER) to form triglycerides. Proteins are synthesized in the rough endoplasmic reticulum (RER). The proteins combine with the triglycerides in the Golgi bodies to form **lipoproteins**, which are packaged into small membrane-bound structures, prior to secretion to the blood.

Bile secretion

Bile secretion begins in the fetus from about week 12 of gestation.
The components of bile are water and **bile salts** (**sodium glycocholate** and **sodium taurocholate**). The bile, which is excreted via the bile duct to the duodenum, is responsible for the emulsification of fats. Synthesis of the bile salts takes place in the SER, where there is conjugation of the amino acids glycine and taurine with cholic acid.

The bile also serves as a vehicle for the excretion of components of aged erythrocytes. The breakdown of erythrocytes (by Kupffer cells of the liver, or macrophages of the spleen) results in the **heme pigment** being converted to **biliverdin**, which is converted to **bilirubin**. Bilirubin, which is water-insoluble, is conjugated with glucuronic acid in the SER of hepatocytes to form water-soluble **bilirubin glucuronide**, which is secreted in the bile. In the duodenum the bilirubin is converted by bacteria to **urobilinogen**, which is reabsorbed in the distal small intestine and recaptured in the liver. About 90% of the bile is

recycled, with about 10% de novo formation. The bile provides the characteristic dark color of the feces.

Metabolic zones in the hepatic lobules

It is possible to divide the lobules into areas of differing metabolic activity. The most peripheral areas of the lobules, have the richest blood supply (greatest oxygenation, more metabolites). This peripheral area is called the **zone of permanent function**. The middle zone of the lobules has reduced metabolic activities and is known as the **zone of intermediate function**. The most central zone, closest to the central canal, has the least metabolic function and is known as the **zone of permanent repose** or inactivity. This is reflected in the much greater numbers and activities of mitochondria in the peripheral (**perilobular**) zone as compared to the inner (**centrolobular**) zone.

If an experimental animal is well fed, the glycogen accumulates in the hepatocytes of the peripheral zone and only later to a lesser degree in the hepatocytes of the middle zone. The hepatocytes of the inner zone show virtually no accumulation of glycogen. If the animal is then starved, the glycogen first disappears from the middle zone and only later from the most peripheral zone.

Various concepts of lobules

The **classical** (hexagon-shaped) **lobule** differs from the lobules of other exocrine glands, where the main secretory duct is located in the center of the lobule. In the classical lobule, the secretory ducts (bile ductules) are at the periphery.

It is possible to consider a condition similar to other exocrine glands with a central location for secretory ducts in the lobules. This concept is known as the **portal lobule**. However, the portal lobule has no clearly defined borders and can be considered as a triangular structure stretching between three central veins.

A further concept of hepatic lobules is that based on functional areas. In this case the functional unit is described as the **acinus (**of Rappaport). The borders of the acinus are not clearly defined, but are based on physiological areas. The acinus is a diamond-shaped structure in which physiological zones are defined. This concept is important in the understanding of several histopathological conditions.

Regeneration

Parenchymal cells of the liver have a remarkable capacity for regeneration. If up to 60% of the liver is removed surgically from an experimental animal, the liver tissue can regenerate and return to its original dimensions within a relatively short time.

Many chemicals are cytotoxic to liver cells. These include chlorinated hydrocarbons (pesticides) and a range of common organic solvents and anaesthetics (carbon tetrachloride, xylene, chloroform, ether).

THE GALLBLADDER

The function of the gallbladder is to provide a storage site for bile (synthesized in the liver). The bile also becomes more concentrated in the gallbladder owing to ion-transporting activities of the epithelium lining the lumen.

The epithelium lining the lumen is a **simple, columnar, homogeneous epithelium**. The epithelium typically has many folds, however, when the gallbladder is filled with bile, these folds disappear.

The layers of the gallbladder consist of:

1. ***Mucosa*** consisting of columnar epithelium and lamina propria. The cytoplasm of the epithelial cells has weak acidophilic characteristics. The nuclei tend to be oval and situated in the more basal part of the cells.
2. ***Muscularis*** consisting of smooth muscle, which is found in longitudinal, transverse and oblique directions.
3. ***Perimuscular dense connective tissue layer***.
4. ***Serosa,*** which covers part of the organ only.

Lipids reaching the duodenum signal the release of the polypeptide hormone, **cholecystokinin** (**CCK**) from endocrine cells of the mucosa into the blood. The cholecystokinin has receptors in the wall of the gallbladder, which result in the contraction of the smooth muscle and the release of the bile via the bile duct on to the duodenum.

PANCREAS

The pancreas is a compound gland with both exocrine and endocrine functions. Unlike the liver, the exocrine and endocrine functions are from different cells.

THE SKIN

The skin, also known as the ***cutis*** or **integument** serves as the main cover of the body. The skin is considered as the largest organ of the body weighing up to 16% of total body weight.

The skin consists of two main layers:

Epidermis (of ectodermal origin)
Dermis (derived from mesoderm).

The **Hypodermis** is a subcutaneous layer, composed mainly of adipose tissue. The border betwen the epidermis and dermis is distinct and consists of a series of ridges (**epidermal ridges**) and papillae (**dermal papillae**). In thick skin the border is seen to be grooved (parallel to the fingerprints), whereas in thin skin the border consists of pegs and sockets. The border between the dermis and hypodermis is indistinct.

Functions of skin

1. **Mechanical protection**. The stratified keratinous epithelium of the skin provides mechanical protection against external abrasions or injury and against invasion of foreign objects.

2. **Thermoregulation**. The skin plays a major role in regulation of body temperature, in particular by means of arterio-venous anastomoses.

3. **Osmoregulation**. The skin is important in the regulation of body fluids and ions, and also protects against fluid loss.

4. **Excretion and secretion**. The skin possesses exocrine glands (sweat glands, sebaceous glands).

5. **Sensory reception**. The skin receives sensory stimuli from the external environment. Several different types of receptor present in the skin respond to a variety of stimuli such as touch, pressure, heat, cold, pain.

6. **Metabolic functions**. The prohormone of vitamin D is present in the skin and is stimulated by ultraviolet light to provide the initial stage of the pathway leading to the synthesis of vitamin D metabolites.

7. **Absorption**. Various substances, including many drugs, can be absorbed through the skin (transdermal absorption). Encapsulation of drugs in a skin

patch and their slow delivery can result in a continuous and steady absorption. This is common today with nitroglycerine (for cardiac therapy), nicotine (to help addicted cigarette smokers quit), estrogen (for postmenopausal replacement) or drugs to counter motion sickness.

The skin is a very mobile tissue and needs the capability to stretch in areas of the body that are not static. This is well illustrated by considering the skin covering the elbow.

SKIN APPENDAGES

Skin appendages (derived from epithelium) include:

(a) **hair**
(b) **nails**
(c) **exocrine glands** (sweat glands, sebaceous glands)

The free surface of the skin is not smooth, but has a series of fine grooves. In areas of the skin lacking hair these are more distinct. In particular the grooves are most pronounced on the thick skin of the hands (fingerprints) or the feet.

The skin in adults covers a total **surface area** of about $2.2m^2$. The surface area increases approximately sevenfold during the lifetime from an infant to adult and it is important to estimate skin surface in order to calculate dosage of drugs to be administered. The surface : volume ratio in young children is much greater than that of adults and consequently their control of body temperature is more problematic. A child loses heat more rapidly than an adult.

The thickness of the epidermis varies from 0.12-0.17 mm over most surfaces of the body ("**thin skin**"), but may reach a thickness of 0.8 mm on the palms of the hands and up to 1.4mm on the soles of the feet ("**thick skin**"). The terms thin skin and thick skin refer to the epidermis only and not to the total thickness of the skin.

Thick skin (of the palms and soles) is hairless, lacks pigment and is sometimes described as glabrous. Thin skin, in contrast, has hair, sebaceous glands and is pigmented.

The Epidermis

The epidermis consists of **stratified squamous epithelium with a layer of keratin**. The development of this layer of keratin was very important in

evolution as it allowed the emergence of vertebrates from a fluid environment to a terrestrial environment.

4 cell types, with different origins, are distinguished in the epidermis:

1. **Keratinocytes** (keratin production)
2. **Melanocytes** (pigment production)
3. **Langerhans cells** (immune system)
4. **Merkel cells** (diffuse neuroendocrine system).

The Keratin System

Keratinocytes (derived from ectoderm) are the dominant cell type of the epidermis, and are part of the keratinizing system, involved in the **cornification** or **keratinization** of the skin. The most superficial cells are essentially dead cells, or **scales**, primarily composed of keratin. These superficial keratinized cells are continuously being lost (**desquamation** or **exfoliation**) and need to be replaced. New keratinocytes are continuously formed in the basal layers of the epithelium and mitotic figures are commonly seen in these layers. The process of differentiation of the basal cells into the desquamating superficial scales is known as **cytomorphosis** and takes about 15 days in infants and up to 30 days in adults. The epidermal layer is therefore being totally replaced in a relatively short period. The skin disorder, psoriasis, is characterized by a very rapid turnover of keratinocytes (7 days).

The morphological changes occuring in the keratinocytes and the layers are best seen in thick skin.

Epidermis of thick skin

(a) **Basal layer** (*Stratum basalis*)

The epithelial cells of the basal layer are closest to the basal lamina and to the underlying dermis. These cells are typically **columnar or cuboidal** with many examples of mitosis. At the ultrastructural level the most basal cells have unique structures on their basal plasmalemma known as **hemidesmosomes** (like half a desmosome). The cytoplasm of the cells has **tonofibrils** (visible by light microscopy), which are seen to be **tonofilaments** (7-8 nm diameter) at the ultrastructural level. These tonofilaments are characterized as **intermediate filaments** composed of **prekeratins**. As the basal cells continue to develop and move into the more superficial levels, the tonofilaments can constitute up to 50% of the proteins of the cells.

(b) **Spiny layer** (*Stratum spinosum*)

The keratinocytes of the spiny layer are more rounded or oval and at the light microscope level there appear to be pronounced gaps between the cells. The cells appear to be connected by "spines". These spines, seen by ultrastructure, are composed of prominent **desmosomes**. Tonofibrils are very dominant in these cells. Mitoses are also common in the spiny layer.

The basal layer and the spiny layer together are known as the **Malpighian layer** or **germinative layer** (as this is the area of mitoses and generation of new keratinocytes).

(c) **Granular layer** (*Stratum granulosum*)

This layer consists of 2-5 rows of fairly flattened cells characterized by large (1-5μm) cytoplasmic basophilic granules known as **keratohyalin granules**. Keratohyalin consists of phosphorylated histidine-rich proteins and cystine-containing proteins and is the precursor of keratin. These granular cells also **membrane-coating granules**, which secrete their lipid-containing contents by exocytosis on to the surface of the cells. This secretion provides the **water impermeable barrier** of the skin.

(d) **Clear layer** (*Stratum lucidum*)

This is seen as a thin, undulating layer, stained well with eosin. The nuclei of the cells are not well distinguished. The cytoplasm of the cells contains a substance called **eleidin**, apparently derived from keratohyalin. Relatively little is known about eleidin or its significance.

(e) **Horny layer** (*Stratum corneum*)

The cells of the horny layer are flattened and their nuclei can no longer be distinguished. The cells show a thickening of their plasmalemma (up to 15 nm). The cells are packed with keratin. **Keratin** belongs to the **scleroproteins**. The keratin molecule is rich in disulfide bonds and is anisotropic (birefringent) when examined by polarizing microscopy.

Epidermis of thin skin

The epidermis of thin skin is much thinner and less well developed than that of thick skin. The basal layers and horny layers are always present, but much

thinner, however, the clear layer is usually not found and the granular layer is very thin, consisting of a single or double row of cells.

Skin cancer derived from basal layers is known as **basal cell carcinoma**, whereas skin cancer developing from more superficial cells is known as **squamous cell carcinoma**. These skin cancers are extremely common and constitute up to 30% of all the tumors of the body. They are usually not life-threatening and relatively easy to treat.

The Pigmentary System

The color of the skin depends on its thickness, and the degree of underlying vascularization, especially the oxyhaemoglobin. If blood vessels are contracted, such as in cold environments, the skin is more pallid, whereas if we exert ourselves the vessels dilate and we appear redder. The yellowish color of skin is due to the pigment carotene (excessive dietary intake of carotene-containing foods may induce a more yellow or orange coloration). Skin color can sometimes provide diagnostic clues to underlying disorders (anemia, cyanosis, hepatitis).

The main pigment of the skin, which provides the main component of skin coloration, is **melanin**. Melanin is produced by **melanocytes**, derived from the embryonic **neural crest**. Melanocytes are found in the more basal layers of the epidermis. They are rounded cells with processes that extend between the adjacent keratinocytes. They are not connected to the keratinocytes by any structures and lack desmosomes. The melanocytes need to synthesize the enzyme, **tyrosinase**, in order to synthesize melanin. **Albinos** have an inborn error of metabolism and are unable to synthesize tyrosinase and are consequently unable to produce melanin.

Melanin is lacking in thick skin. The palms of the hands and the soles of the feet are unpigmented, even in dark-skinned people.

The synthetic process of melanin formation within the melanocytes involves a simple biosynthetic pathway:

Tyrosine is converted (by tyrosinase) to DOPA (dihydroxyphenyl alanine) and DOPA-quinone, which is converted to the dark pigment melanin.

The melanin is seen in histological sections as a dark brown color in both melanocytes and adjacent keratinocytes. The melanin is transferred to the keratinocytes (apparently by phagocytosis) and as a result more melanin may accumulate in the keratinocytes than in the melanocytes. The development of

melanin granule formation at the ultrastructural level reveals rugby-ball shaped bodies formed by the Golgi bodies. These initially have little pigment and are known as **premelanosomes**, but soon become packed with melanin and are known as **melanosomes**. It is the melanosomes (melanin granules) that are taken up by the keratinocytes.

The number of melanocytes is fairly constant in all races (about 1000/mm^2 on most of the body, but 2000/mm^2 on the scrotal skin). The difference between races is in the rate of expression of melanin formation, being much greater in people with dark skins.

Skin pigmentation is genetically determined, though in lighter-skinned people pigmentary changes commonly result from (a) endocrine disturbances (such as excess ACTH) (b) pregnancy (including changes in the color of the areola of the nipples) (c) exposure to ultraviolet light ("suntan").

The **tanning process** following exposure to sunlight is a protective measure to reduce the damage of ultraviolet light to the underlying tissues. Following initial inflammatory changes, there is increased synthesis of tyrosinase and melanin. Ultraviolet light is also a cause of age-related skin changes, including skin thickening, hardening and increased wrinkling.

Malignant melanoma is a serious form of skin cancer that develops from proliferation of melanocytes. Excess ultraviolet light exposure appears to be a predisposing factor in the development of melanoma.

Langerhans cells

Langerhans cells are found in the epidermis and number 400-1000 per mm^2. They are star-shaped cells found mainly in the spiny layer and can be demonstrated by impregnation with gold chloride techniques. They function as **antigen-presenting cells of the immune system** of the skin. At the ultrastructural level their cytoplasm is seen to possess characteristic rod-like granules known as **Birbeck's granules**.

Merkel cells

These are found in thick skin and at the ultrastructural level are seen to possess numerous cytoplasmic granules, typical of polypeptide-secreting endocrine cells. These cells are commonly associated with free nerve endings. The function of Merkel cells is still not fully established, though it is thought they may function as sensory mechanoreceptors or produce local neuroendocrine

secretions. It is believed that they belong to the diffuse neuroendocrine system of the body.

The Dermis

The **dermis** is the layer of connective tissue beneath the epidermis and is derived from mesoderm. It is difficult to measure the thickness of the dermis as the border with the underlying hypodermis is not distinct. Over most of the body the dermis is 1-2 mm thick, though in thick skin it may reach 3 mm or more. The dermis of the dorsal side of the body is in general thicker than that of the ventral side. Usually the dermis is thinner in females than in males.

Two distinct regions are present in the dermis

(a) **papillary layer**
(b) **reticular layer**

The **papillary layer** includes the area of the dermal papilla and consists of **loose connective tissue**. The main blood vessels that supply the epidermis are located in the papilla. (As the epidermis is avascular, the location of the blood vessels in the papillae reduces the distance needed for diffusion).

The dermis is well-vascularized and has many **arterio-venous anastomoses**, which play important roles in thermoregulation of the body. Up to 4.5% of blood volume is found in the dermis. In cold external environments the body needs to conserve heat and the blood can go directly from the arteries to the veins, by-passing the capillary bed. In hot environments, when we need to cool the body more, the blood is allowed to pass into the capillary beds.

The **reticular layer** is thicker than the papillary layer and consists of a more dense, irregular connective tissue composed mainly of collagen bundles. The term reticular refers to the netlike arrangement of these bundles.

Both the papillary and the reticular layers have **elastic fibers**, responsible for the elasticity and flexibility of the skin. With aging the collagen fibers thicken and cross-link and the elastic fibers lose much of their elasticity, causing increased wrinkling of the skin. The process of **age-related wrinkling** is increased in cigarette smokers and in people exposed excessively to ultraviolet light. Sun exposure is a major factor in age-related damage to skin.

The dermis of the face is the site of insertion of the **muscles of facial expression**. These striated muscles are vestiges of a more extensive

subcutaneous muscle layer found in many mammals (***Panniculus carnosus***), which is used to shake wet fur dry, or dislodge insects.

Several epithelial derivatives are also found in the dermis (hair, sweat glands, sebaceous glands).

Several types of **sensory receptors** are also found in the dermis.
Meissner's corpuscles are tactile mechanoreceptors found in dermal papilla, especially in thick skin, lips, eyelids, genitalia, nipples.
Pacinian corpuscles (Vater-Pacini) are mechanoreceptors common in the dermis of thick skin.

The Hypodermis

The hypodermis consists of loose connective tissue and is composed mainly of adipose tissue.

SKIN APPENDAGES

Hairs

Hairs are one of the unique characteristics of mammals. They are thin filaments of keratin that develop in the dermis from epithelial invaginations of the epidermis, which interact with a germinative center of the dermis - the dermal papilla.

Hairs are found on skin of most parts of the body, apart from thick skin (palms of the hands, soles of the feet), lips, glans penis, labia minora and clitoris. The number of hairs on the face are about 600 hairs per cm^2, whereas in the other parts of the body they are about 60 per cm^2.

The appearance of hairs (length, thickness, pigmentation, density) depends on many factors including: age, race, sex, hormones, location on the skin. Hairs grow discontinuously, with periods of growth followed by periods of inactivity. The hairs on the head may have a growing phase of 3 years or more, whereas in other areas of the body the growing phase may be only 3 months or so. Hair growth is not synchronous and in any particular site, there will be hairs in active growth phases as well as hairs in inactive phases. This is described as mosaic growth.

The development of hairs involves epithelial invaginations (proliferation and downgrowth) from the epidermis into the connective tissue of the dermis. The

tubular epidermal invagination is known as the **hair follicle**. The most terminal portion of the invagination expands to form the **hair bulb**.

A connective tissue sheath surrounds the follicle (**dermal sheath**). During development there is a process of induction during which a concentration of connective tissue (**dermal papilla**) becomes associated with and surrounded by the hair bulb. The association of the dermal papilla and the hair bulb is essential for hair growth. Blood vessels invade the dermal papilla in order to supply the necessary nutrients and hormones for hair growth.

During hair development two epithelial protrusions develop on the follicle. The upper protrusion is the site for the development of the **sebaceous gland**. The lower protrusion is the site for attachment of a smooth muscle bundle, known as the **arrector pili**. The site of insertion of the arrector pili muscle is in the papillary layer of the dermis. Contraction of the arrector pili muscles causes extrusion of the oily secretion (sebum) from the sebaceous glands, via a short duct onto the surface of the growing hair. The arrector pili muscles contract in response to fear, anger or cold and are responsible for gooseflesh.

The **germinative area** of the hair is a short cone-like region just above the hair bulb. This is the region where the hair cells become keratinized and pigmented.

When examined in more detail, the epithelial components of the hair are divided into an **outer epithelial sheath**, continuous with the Malphighian layer of the epidermis and an **inner epithelial sheath**, which extends only to the level of the sebaceous glands. The epithelial cells of the outer epithelial sheath grow downwards in the direction of the bulb and continue upwards in the inner epithelial sheath.

The hairs have a central core (**medulla**) composed of large vacuolated cells, with relatively little pigment or keratin. This is absent in the fine **lanugo** hairs of the newborn, that are usually shed within a month or so. The medulla is surrounded by the **cortex**, composed of cells that are usually very pigmented and keratinized. The outermost part of the hair is the **cuticle**, which is composed of overlapping scales of hard, transparent (non-pigmented) keratin.

Melanocytes, situated in the hair bulb, transfer their pigment to the cells of the medulla and cortex. With aging the melanocytes produce less melanin resulting in white hair. The part of the hair that projects from the skin consists of non-viable (dead) cells.

The outer epithelial sheath lies on a thick basal lamina (the "**glassy membrane**"), which separates the follicle from the surrounding dermal sheath.

During periods of cessation of hair growth, the hair papilla becomes detached from the hair bulb and the hair follicle adopts a club-like appearance (**club hair**). It is during these periods that hair loss is experienced. Hair growth is influenced by endocrine factors and in particular the sex steroids. The changes in hair distribution associated with sexual maturity are the result of changes in endocrine secretion and development of receptors to androgens in particular.

Sebaceous glands

The sebaceous glands are dermal exocrine glands associated with hairs and which secrete an oily substance (**sebum**) on the growing hair. The sebum is important in maintaining the flexibility of the hair. The glands are composed of alveoli and a short secretory duct. The outermost cells of the gland are filled with lipid droplets. These cells are secreted in their entirety (**holocrine secretion**). The extrusion of the sebum results from the contraction of the arrector pili muscles. The sebum secretion is influenced by sex hormones (androgens and estrogens) and during hormonal imbalance during puberty may result in adolescent acne.

Sebaceous glands are not present in thick skin.

Sweat glands

These are simple exocrine glands that secrete sweat. The **secretory units** are simple convoluted tubular epithelial structures in the dermis and a straight **secretory duct**. The convolutions of the secretory unit are seen in histological preparations as many associated profiles of the same unit. The secretory ducts are surrounded by **myoepithelial cells**, which on contraction cause the expulsion of the sweat.

Two sorts of sweat glands are found:

(1) **Eccrine (Merocrine) sweat glands**

These are found all over the body including the thick skin. In thick skin sweat pores are conspicuous in the thick outer layer of keratin. Fingerprints are formed by secretions derived from the sweat glands. The eccrine sweat glands have cholinergic innervation.

(2) **Apocrine sweat glands**

These are large sweat glands located in the axilla (armpit) and also in association with the external genitalia and anus. They have very large secretory units and myoepithelial cells, in which the apical part of the secretory cells including their contents, are secreted into the lumen (**apocrine secretion**). The breakdown of this secretion by bacteria is the cause of the typical smell of sweat from the armpits. The apocrine sweat glands only become functional at puberty and are influenced by sex steroids. (Young children do not have a noticeable sweat smell.) It is possible that the secretions of apocrine sweat glands contain pheromones (as sex chemoattractants). The apocrine sweat glands have adrenergic innervation.

Nails

Nails are horny plates of keratin on the dorsal surface of the terminal phalanges of the hand and foot (fingers and toes).

The nail plate is composed of tough, hard, clear non-desquamating keratin, which sits on the **nail bed**, composed of a layer of epidermal cells. Surrounding the nail is the **nail groove**. The formation of the nail and its keratin occurs in the **nail root**. This region shows many mitoses of the epithelial cells. These germinative cells of the nail root are known as the **dorsal and ventral matrix**. Part of the ventral matrix (seen as a whitish half-moon near the dorsal nail groove) is called the **lunula**. The keratin above the nail groove is thickened (**eponychium**). Below the nail, near its free surface, the keratin is also thickened (**hyponychium**).

If the terminal portion of a finger is cut transversely, the lateral nail grooves are easily seen, and the clear nail plate is also obvious. The nail bed, however, is seen to have many small undulations, in which small blood vessels and nerves are located.

Nerve endings in the skin

The skin has many sensory elements that respond to external impulses and signals.

(1) **Free nerve endings** are non-encapsulated nerve endings in the epidermis and which respond to pain.

(2) **Meissner corpuscles** are mechanoreceptors present in the dermal papilla.

(3) **Merkel corpuscles** are mechanoreceptors surrounding hair follicles responsive to touch.

(4) **Pacinian corpuscles** (Vater-Pacini) are found in the dermis of thick skin of fingers and respond to pressure and vibration.

(5) **Krause end bulbs** are found in the dermis and respond to cold.

Split skin grafts

In cases of trauma or burns in which the skin is damaged, skin transplants (autografts) can be taken to cover the wounds. In these cases a thin slice of skin is removed (epidermis and dermis) from an uninjured region and transplanted to cover the lesion. The epithelium of the skin rapidly proliferates to cover the wound and the epithelial components (hairs and glands) also rapidly recover. The area from where the graft was taken also rapidly recovers. The epidermal derivatives (hair and glands) rapidly grow outwards to form new epidermis.

RESPIRATORY SYSTEM

The function of the respiratory system is to bring oxygen to the blood and to remove the carbon dioxide. The system is composed of two parts:

(a) **Conducting Portion**.
(b) **Respiratory Portion**.

The **Conducting Portion** consists of a series of cavities and tubes conducting air to the lungs. It is composed of :

Nose
Nasopharynx
Larynx
Trachea
Bronchi
Bronchioles (terminal and respiratory). The respiratory bronchioles constitute an area of transition between the conducting and respiratory systems.

Some of these structures lie outside the lungs (**extrapulmonary**) and need cartilaginous supports in their walls, which provide rigidity and flexibility. Some of the structures are inside the lungs (**intrapulmonary**), where the need for structural support is less.

The **Respiratory Portion** consists of:

Alveolar ducts
Alveolar sacs
Alveoli.

The exchanges of gases (respiration) only occurs in the alveoli.

The **functions of the conducting portion** are to provide a route for the air to reach the lungs and also for conditioning the air. Air-conditioning during its passage through the conducting portion includes:

filtration (by hairs)
cleansing (by mucus and ciliary action)
moistening (by mucus)
warming or cooling (by heat exchange via blood vessels).

These air-conditioning functions are performed by a specialized epithelium, the "**respiratory epithelium**", which lines much of the surface of the conducting portion.

Respiratory epithelium is a **pseudostratified columnar ciliated epithelium with goblet cells**. The epithelium lining the smaller tubes of the bronchial tree becomes simple cuboidal epithelium. The goblet cells of the respiratory epithelium secrete mucus, which traps dust and particulate matter, which is propelled by the ciliary action towards the pharynx. This mucus moistens and lubricates the ciliary surface and provides a barrier to prevent much of the dust entering the lungs. The amount of mucus increases greatly in cases of infections of the respiratory tract.

NOSE AND NASAL CAVITY

The external nostrils (**nares**) and **vestibule** have coarse hairs to filter large particles. The nose is divided by the **nasal septum** into two chambers. Three incomplete plates of bone (**conchae** or **turbinates**) divide each chamber into three smaller chambers (superior, middle and inferior). The surface of these chambers is lined with respiratory epithelium, apart from the superior chamber, which is lined by a specialized olfactory (smell) receptor (***Regio olfactoria***).

Regio olfactoria

The olfactory region (about 10 cm^2) is lined by a highly specialized sensory "epithelium", which is in fact composed of neurons and glia. This is unique as neurons are usually not in direct contact with the external environment.

The components of the olfactory "epithelium" consist of :

(1) **Olfactory cells**. These are **bipolar neurons**. The apical part of the neuron is a modified dendrite, which has a terminal globular vesicle (**olfactory vesicle**) with 6-8 non-motile cilia, which are very long (150-200μm). The **perikaryon** is located in the middle of the "epithelium" and leads to the axon, which enters the underlying connective tissue. The axons in this connective tissue are present in small bundles (***fila olfactoria***) surrounded by an envelope of dense connective tissue. These axons are non-myelinated, lack endoneurial sheaths, and belong to the central nervous system.

(2) **Supporting cells**. These are columnar cells (really glia) and contain a yellow pigment in their cytoplasm.

(3) **Basal cells**. These are small cells situated close to the basal lamina. It is possible that these are replacement cells for the supporting cells.

The ***lamina propria***, in addition to the *fila olfactoria*, possesses epithelial tubulo-alveolar **glands of Bowman**. These produce secretions that are conveyed to the free surface of the epithelium via secretory ducts. These secretions are believed to function to moisten and refresh the sensory surface of the chemoreceptors.

Most food that we eat is "tasted" by chemoreception of the olfactory organ. The taste buds of the tongue can only distinguish between sweet, bitter, acid and salty. People suffering from colds are unable to distinguish the aromas of food and typically do not have an appetite.

The ***lamina propria*** of the conchae, including those of the *Regio olfactoria*, possesses large **vascular venous plexuses**. These blood vessels are thin-walled and possess both longitudinal and circular smooth muscle. These vessels can engorge with blood (similar to erectile vessels of the penis). Erotic stimulation can cause engorgement. Many animals, such as dogs, where the sense of smell is much greater than that of humans, have much more extensive olfactory areas and typically use their olfactory sense to detect hormonal secretions in other dogs. It is possible that in humans this function no longer plays a significant role as we hide our natural smells by bathing and use of deodorants.

These blood vessels (also known as "**swell bodies**") engorge periodically on alternate sides of the nose to reduce the rate of airflow and protect the epithelium from desiccation.

A further function for the rich vascular blood system of the *lamina propria* is to warm inspired air, especially in cold climates.

The nose leads to the **nasopharynx**, the first part of the pharynx. The development of the palate in evolution was of utmost biological significance as it enabled the separation of the route for air (nasopharynx) from the route for feeding (oropharynx). People with incomplete palates (cleft palate) suffer from many clinical problems and palate malformations need to be surgically corrected as soon as possible.

THE EPIGLOTTIS

The epiglottis is a flexible flap-like structure forming the uppermost part of the larynx. It is thought to have a passive role in preventing food or fluids from entering the larynx.

The epiglottis is lined by **stratified squamous epithelium**. The epithelium of the upper (lingual) surface is usually thicker, than the lower (laryngeal) surface, which continues as respiratory epithelium. The main support for the epiglottis is a plate of **elastic cartilage**, surrounded by perichondrium. Small **exocrine glands** are common in the lamina propria.

THE LARYNX

The larynx is an irregular tube connecting the pharynx to the trachea. The larynx has two functions:

(1) **phonation** (creation of sounds for speech).

(2) **controlling the air pathway** so that only air (and not food or foreign objects) reaches the lower respiratory passages. During swallowing the larynx moves upwards and directs the food to the esophagus. If anything other than air enters the larynx there is a **cough reflex** (responding to fluids or food to prevent them entering the trachea). In cases of drowning, the cough reflex may cause uncontrolled laryngeal spasm, preventing oxygen reaching the lungs and death by asphyxiation. Autopsies of bodies after drowning commonly reveal lungs that are virtually free of water.

The mucosa of the larynx has two pairs of folds. The **false vocal cords (**upper folds) are separated from the **true vocal cords (**lower folds) by the **laryngeal ventricle**. The true vocal cords, by modifying the slit-like opening (***rima glottidis***) enable us to produce sounds.

The **true vocal cords** consist of:

(1) **stratified squamous epithelium**
(2) **vocal ligament** (connective tissue, which is mainly elastic bundles)
(3) **vocal muscle** (skeletal muscle, which regulates the tension of the folds).

The **false vocal cords** consist of:
(1) **respiratory epithelium**
(2) ***lamina propria*** with many exocrine glands

Irregular plates of **hyaline cartilage** provide support and protection for the larynx.

Lymphatic nodules are common in the *lamina propria* of the larynx, especially in the area of the false vocal cords.

TRACHEA

The trachea is a short tube (about 10 cm long) extending from the larynx to the bifurcation at the beginning of the two primary bronchi. The trachea is lined with typical respiratory epithelium. In the *lamina propria* there are about 20 **C-shaped rings of hyaline cartilage** (the open ends are posterior). The open ends of each ring are connected by a bundle of **smooth muscle**. A **dense fibroelastic ligament** continuous with the perichondrium of each ring connects adjacent rings. This ligament prevents overdistention of the lumen. In histological preparations as a result of contraction of the smooth muscle, the muscle may be displaced and the C-shape of the rings may be distorted.

BRONCHIAL TREE

The tubes bringing air to the lungs continually divide into smaller tubes (trachea, primary bronchi, secondary bronchi, terminal bronchioles) and are often described as the bronchial tree. In humans this has the general appearance of an oak tree. The bronchial tree can be well demonstrated if latex casts are made of the respiratory tubes and ducts.

BRONCHI

The trachea divides into the two **primary bronchi**, which enter the lung at the **hilum**. These primary bronchi divide into smaller **secondary bronchi** (3 in the right lung, 2 in the left lung). The **extrapulmonary bronchi** have a similar histological appearance to that of the trachea. The **intrapulmonary bronchi** (lobar bronchi) have irregular plates of hyaline cartilage (instead of C-shaped rings). In transverse section these appear as small oval or crescent-shaped plates or islands of cartilage. As the bronchi become smaller, the bands of smooth muscle in the wall become more prominent. Contraction of this smooth muscle after death typically causes the mucous membrane of the bronchi to appear to have longitudinal folds. The *lamina propria* of the bronchi has abundant elastic fibers.

BRONCHIOLES

Terminal bronchioles

The first and larger bronchioles are the **terminal bronchioles**. These have a diameter less than 1mm and lack cartilage in their walls. They lack glands in the mucosa. Goblet cells are virtually absent and if present are only found in very small numbers in the initial segments. A simple ciliated columnar or cuboidal epithelium

lines the terminal bronchioles. (In the initial segments of the largest bronchioles, it may be pseudostratified). **Clara cells** (non-ciliated secretory cells) are dome-like cells located between the ciliated cells. The function of Clara cells is still unknown. They secrete glycosaminoglycans in response to chemical irritation (xenobiotics).

The *lamina propria* of the terminal bronchioles contains relative large amounts of smooth muscle and elastic fibers. These smooth muscles contract and severely restrict air-flow during asthmatic attacks. Asthmatics use drugs that stimulate the sympathetic innervation of the smooth muscle and cause their relaxation resulting in distention of the bronchiolar diameter.

Respiratory bronchioles

These are short tubes regarded as areas of transition between the conducting and respiratory portions of the respiratory system. The diameter of the respiratory bronchioles is about 0.5mm. The lining epithelium is simple cuboidal and non-ciliated. They have no cartilage in their walls. They lack goblet cells.

Respiratory portion of the lung

The functional unit of the lung (primary lobule) includes the :

- **Alveolar ducts**
- **Alveolar sacs**
- **Alveoli**

These form sponge-like, elastic, thin-walled air-filled structures with extensive capillary beds, where respiratory gas exchange occurs.

The **alveolar ducts** are short tubes into which open numerous alveoli. "Knobs" of smooth muscle can be seen separating adjacent alveoli. The only support for the alveolar ducts and alveoli is a rich matrix of elastic and collagen fibers.
The terminal part of the alveolar duct opens into an atrium (**alveolar sac**) into which open many alveoli. The sac-like **alveoli** are the final elements of the bronchial tree. Each alveolar sac is surrounded by a rich network of blood capillaries. It has been estimated that each adult lung has about 300 million alveoli, with a total surface area for gas exchange of 70-80 square meters.

Adjacent alveoli may be connected by small openings with diameters of about 10-15um (**alveolar pores**). These pores may help equalize air pressure in adjacent alveoli.

The alveolar "wall"

The alveolar "wall" is composed of three main cell types :

- **Endothelial cells** of blood capillaries (continuous, non-fenestrated)

- **Squamous epithelial cells** (Type I)
- **Secretory cells** (Type II, great alveolar cells)

The alveolar "wall" is extremely thin. The total thickness of the alveolar "wall" for gas exchange is only about 0.2-0.6um. A common basal lamina is found between the alveolar cells and the endothelial cells.
The **squamous epithelial cells**, which constitute the main cell type of the alveoli, are as the name implies extremely thin.
The **alveolar secretory cells** (Type II) are large rounded cells, which synthesize and secrete **surfactant**. Surfactant is a phospholipid (dipalmitoyl lecithin), which reduces the surface tension of alveoli. Without surfactant the alveoli collapse and cannot function. Surfactant is only produced and secreted towards the end of gestation. Premature babies lack surfactant and suffer from **respiratory distress syndrome**. At the ultrastructural level the surfactant is seen in the type II cells as multilamellar bodies (about 0.2um diameter).

Alveolar macrophages

Alveolar macrophages ("**dust cells**") are mobile phagocytic cells in the lung that seek and collect particulate matter that has managed to reach the lungs. These belong to the Mononuclear Phagocyte System. Alveolar macrophages are much more prominent in lungs or smokers or people from cities with industrial pollution.

URINARY SYSTEM

The urinary system consists of:

Kidneys (2)
Ureters (2)
Urinary bladder
Urethra

KIDNEYS

Functions of the kidneys include:

1. **Urine production**

2. **Excretion of metabolic waste**

3. **Homeostasis of body fluids** (regulation of total body water and volume of extracellular fluid)

4. **Homeostasis of electrolytes**

5. **Hormone production** :

(a) active metabolites of vitamin D3
(b) renin
(c) erythrogenin (precursor of erythropoietin)

6. **Control of acid-base balance** (pH of the body)

7. **Role in blood pressure control**.

Renal Morphology (Macroscopic)

The kidneys are **bean-shaped retroperitoneal structures**. The area of the concave surface of the kidney where the nerves, blood and lymph vessels enter and leave is known as the **hilum** (or hilus). The kidney is covered with a **capsule** composed of dense connective tissue. If a whole kidney is sliced vertically it can be seen to be composed of an outer **cortex** and an inner **medulla**. The main structures seen in the medulla are 10-18 **medullary pyramids**. The areas of connective tissue between the pyramids are known as **Columns of Bertin**. (This is cortical tissue situated in the medulla).

The upper expanded area of the ureter is known as the **renal pelvis**. This further divides into 3 or 4 **major calyces**, each of which subdivides into 2-3 **minor calyces**. The apex of each medullary pyramid fits into a minor calyx and has 10-25 openings through which the urine produced in the kidney is excreted before passing through the ureter to accumulate in the urinary bladder.

From the base of each pyramid, 400-500 **medullary rays** extend radially into the cortex. These medullary rays are straight collecting ducts into which nephrons open.

Renal lobes are defined as a medullary pyramid and the surrounding connective tissue. The human kidney is described as being **multilobar** (10-18 lobes). **Renal lobules** are defined as single medullary rays and surrounding tissue i.e. all the structural elements that drain into a common collecting duct.

The **morphofunctional unit of the kidney is the nephron**. There are an estimated 1.3 million nephrons in each kidney. Each nephron is about 50-55 mm long and it is estimated that the total length of all the nephrons of both kidneys is about 100 km.

The nephron

Each nephron consists of:

1. **Renal corpuscle** (Renal body, Malpighian body)
2. **Proximal convoluted tubule**
3. **Loop of Henle**
4. **Distal convoluted tubule**
5. **Collecting duct** (Some authors do not include the collecting duct as a part of the nephron owing to its different embryologic origin).

All the components of the nephron are epithelial structures, with a continuous basal lamina and surrounded by a delicate reticular fiber network.

1. **Renal corpuscles** (Malpighian bodies)

The renal corpuscles (renal bodies) are round or ovoid structures (about 150-200μm diameter) located in the cortex. They have a double-walled epithelial capsule (**Bowman's capsule**). The outer or **parietal layer of Bowman's capsule** is composed of very flattened (squamous) epithelial cells. The inner or **visceral layer of Bowman's capsule** is composed of specialized epithelial cells known as **podocytes**. An **afferent arteriole** enters the renal corpuscle at the **vascular pole** and forms a complex capillary bed (**glomerular tuft**), before

leaving the **vascular pole** as an **efferent arteriole**. The podocytes are associated with and surround the capillaries of the glomerular tuft and form part of the filtering apparatus.

The structure of the **podocytes** can only be properly visualized by electron microscopy. The podocytes are specialized epithelial cells with a large cell body, the site of the nucleus, and cytoplasmic processes. The **primary processes** divide into smaller **secondary processes**. The processes are known as **pedicels**, which contain abundant microfilaments and microtubules. The secondary processes have numerous fingerlike projections that interdigitate with similar projections from adjacent processes to form **filtration slits** (about 25μm). A thin diaphragm (similar to that of fenestrated endothelium) is found between the slits. The secondary processes lie on a thickened basal lamina (about 0.1μm), which is common with that of the underlying **fenestrated capillaries** (large fenestrae 70-90nm without diaphragms). The basal lamina contains **Type IV collagen** (non-fibrous) and laminin (lamina densa) with associated heparan sulfate (glycosaminoglycan). During filtration, particles larger than 10nm or negatively-charged proteins larger than albumin (MW 69,000) do not pass the filter. The heparan sulfate impedes the passage of negatively charged proteins across the filter.

The fluid that passes from the capillaries through the filter into the capsular space is known as **ultrafiltrate**, which enters the start of the proximal convoluted tubule at the **urinary pole**.

Mesangial cells are cells associated with the capillaries of the glomerulus. They are found in sites where the basal lamina forms a common sheath shared by two or more capillaries. It is thought that mesangial cells may function as a sort of **pericyte**, or may function as **macrophages**, which engulf particulate matter by phagocytosis and help to keep the filter clean. In recent years mesangial cells have also been considered to be contractile and to be specialized **vascular smooth muscle** rather than connective tissue cells.

Glomeruli are commonly classified into two categories according to their location in the cortex (a) **capsular glomeruli** (closer to the capsule) (b) **juxtamedullary glomeruli** (closer to the medulla).

2. **Proximal Convoluted Tubules**

These tubules originate at the **urinary pole** of the renal bodies. They consist of **simple cuboidal epithelium**, which is very **acidophilic** (owing to the activity of the numerous mitochondria). These epithelial cells are relatively large. Their nuclei are regular and fairly central. The apical membrane of each cell is

covered in large numbers of **microvilli** to form a distinct "**brush border**", which is coated with a PAS-positive **glycocalyx**. The lateral membranes of the cells are modified to form numerous **lateral processes**, which interdigitate with similar processes from adjacent cells. The basal region of the cells have many invaginations of the basal plasmalemma. Numerous large mitochondria are situated between the **basal invaginations**. These mitochondria and invaginations result in **basal striations**, which are very well seen in histological preparations stained with iron hematoxylin. **Lysosomes** and **peroxisomes** are common in the apical regions of the cells.

The cells of the proximal convoluted tubules are involved in active **ion-transport**, with large energy demands. In particular they are involved in reabsorption of water and other components of the ultrafiltrate.

3. **Loops of Henle**

Each proximal convoluted tubule continues as the **descending thick segment** of the **Loop of Henle**, which continues as the **thin segment**. The thin segment of the loop is characterized by its extremely flattened epithelial cells, which possess relatively few organelles. The site of the U-shaped loop is an area of extremely concentrated urine. The loop of Henle continues as an **ascending thick segment**.

4. **Distal Convoluted Tubules**

The epithelial cells of the distal convoluted tubule are smaller and less acidophilic (fewer mitochondria) than the proximal convoluted tubule cells. They lack basal striations and also lack brush borders on their apical surfaces.

Juxtaglomerular Apparatus (JGA)

Each distal convoluted tubule comes into contact with the vascular pole of the renal corpuscle of its own nephron and with the afferent arterioles. At this site the distal tubule cells become modified to form a ***macula densa***. The ***macula densa*** consists of tall (columnar) epithelial cells with the most prominent feature being their tall and elongated nuclei. The function of the *macula densa* is unknown though it is thought to transfer data concerning the osmolality of the fluid in the distal convoluted duct.

At the site of contact with the *macula densa* the smooth muscle cells of the *Tunica media* of the afferent arterioles have been modified and become rounded and packed with cytoplasmic granules. These cells, known as **juxtaglomerular cells**, are described as epithelioid (epithelium-like) and are the site of synthesis

and accumulation of the peptide hormone, **renin**. Renin plays an important role in control of sodium and blood pressure. Sodium deficiency (low salt) causes renin secretion (part of the Renin-Angiotensin-Aldosterone hormonal pathway).

Extraglomerular mesangial cells (**Lacis cells**) are also found in the juxtaglomerular region, though their function is still unknown.

5. **Collecting tubules**

In the cortex these are seen as the **medullary rays**, but the collecting tubules dominate in the medulla (in the pyramids). They consist of straight tubules of **cuboidal epithelium** with central nuclei. The cells stain weakly and the borders between adjacent cells are distinct. Near the apex of the pyramid the cuboidal epithelium becomes a **columnar epithelium** and the ducts are much larger and typically ovoid. These are known as the **papillary ducts of Bellini**, and open into the minor calyces.

BLOOD CIRCULATION OF THE KIDNEY

Arterial system:

1. **Renal artery** (elastic artery from aorta, enters at hilum)
2. **Interlobar arteries** (between the lobes in the columns of Bertin)
3. **Arcuate arteries** (arched vessels at cortico-medullary junction)
4. **Interlobular arteries** (in cortex, parallel to medullary rays)
5. **Afferent arterioles**
6. **Glomerular tuft** (and filter in renal corpuscle)
7. **Efferent arterioles** (surround proximal and distal tubules for reabsorption)

Venous system:

The venous vessels accompany the arterial vessels.

1. **Interlobular veins**
2. **Arcuate veins**
3. **Interlobar veins**
4. **Renal vein** (at hilum).

The pyramids of the medulla are composed mainly of straight collecting tubules. Each tubule is accompanied by straight arterioles and venules (known as ***vasa recta***) that originate in the arcuate arteries and return to the arcuate veins.

THE URETER

The ureters are fibromuscular tubes that conduct the urine to the urinary bladder.

They are composed of three layers:

1. **Mucosa**
(a) transitional epithelium
(b) lamina propria

2. **Muscularis**
(a) inner longitudinal muscle
(b) outer circular muscle
(This muscle arrangement is the opposite of that found in the gut)

3. **Adventitia**
Fibroelastic connective tissue.

After fixation, the mucosa is usually thrown into longitudinal folds giving a star-like appearance when seen in transverse sections.

URINARY BLADDER

1. **Mucosa**
(a) transitional epithelium
(b) lamina propria

2. **Muscularis**
Well-developed thick muscular coat of smooth muscle in three layers (hard to distinguish from each other)

3. **Serosa**

URETHRA

The urethra is the urinary tube leading from the bladder to the external orifice (in the male via the penis, in the female it is a fairly straight short muscular tube, 2-6cm)

1. **Mucosa**
Most of the urethra has stratified or pseudostratified columnar epithelium. Transitional epithelium is only found near the bladder.

2. **Muscularis**

The muscle arrangements are like those of the ureter (inner longitudinal, outer circular).

LYMPHATIC ORGANS

The blood-forming tissues of the body (hematopoietic tissue) is a specialized form of connective tissue and is composed of two major divisions:

(1) **Myeloid tissue** (the red bone marrow)

(2) **Lymphoid (or lymphatic) tissue**.

Lymphatic tissue is characterized by an abundance of lymphocytes. There is no lymphatic tissue in myeloid tissue. Red bone marrow should not be regarded as a true lymphatic tissue as it does not contain an abundant population of lymphocytes, but only the stem cells from which lymphocytes develop. Lymphatic tissue is involved with lymphocyte production and immune responses. Lymphatic tissue has a major function in defending the body from disease and the spread of infection. Traces of foreign proteins (such as from invading microorganisms or cells) can elicit an **immune response**.

There is a constant **recirculation** of lymphocytes (especially T-lymphocytes) between the lymph and blood. The lymphocytes can leave the blood and enter the lymph system in specialized blood vessels (**post-capillary venules**) in lymph nodes. They eventually return to the blood together with the lymph via the thoracic duct. This recirculation involves lymphocytes that have acquired the ability to distinguish which molecules or cells belong to the body ("self") from foreign molecules or cells ("non-self"). These recirculating lymphocytes can be considered as being on protective patrol duties and are described as **immunocompetent**. When they encounter foreign molecules or cells they activate a series of immune responses to neutralize and destroy the invader.

TYPES OF LYMPHATIC TISSUE

There are three basic types of lymphatic (lymphoid) tissue:

(1) **Loose lymphatic tissue**. This is dominated by reticular cells and fibers and forms a loose, spongy network through which lymph flow is slow and cells are temporarily trapped.

(2) **Dense lymphatic tissue**. This tissue is dominated by large populations of closely aggregated lymphocytes.

(3) **Nodular lymphatic tissue** (lymphatic nodules). Lymphatic nodules are found in all the lymphatic organs apart from the thymus.

Lymphatic nodules

Lymphatic nodules are found mainly in the loose connective tissue of several organs and in particular in the *lamina propria* of the digestive tract, upper respiratory tract and urinary passages. Nodules are temporary structures, which may appear and disappear in the same site. Nodules are spherical structures (0.2-1mm diameter) lacking a connective tissue capsule. They are mainly composed of dense aggregates of small B-lymphocytes. In many cases the cells at the center of the nodule are much larger, with more cytoplasm. This less-stained central area is known as the **germinal center** and develops according to the functional state of the nodule. Nodules with a germinal center are sometimes described as secondary nodules.

The **germinal center** is the site of proliferation and differentiation of B-lymphocytes. Cell types of the germinal center include: **activated B-lymphocytes** (B-immunoblasts), **mitotic cells, plasma cells, dendritic reticular cells,** and **macrophages**. The ring of small B-lymphocytes surrounding the germinal center is referred to as the **corona** or **mantle zone**. If these small peripheral lymphocytes are aggregated predominantly at one edge, they are referred to as a **cap**.

Newborn individuals, or animals reared in sterile conditions, possess very few lymphatic nodules confirming that these develop in response to exposure to foreign antigens.

Some areas of the body have large aggregations of lymphatic tissue and especially nodules. These include the **Peyer's Patches** of the ileum and the **appendix**.

Origin of lymphocytes

Bone marrow

The **stem cells** from which lymphocytes originate are found in the bone marrow. These stem cells are hard to distinguish morphologically.

CENTRAL LYMPHATIC ORGANS

The stem cells migrate to central lymphatic organs. These are sites of antigen-independent lymphocyte proliferation. Here the lymphocytes acquire their commitment as to whether they will respond to cell-mediated or humoral immune responses. The **thymus** is the site where T-lymphocytes develop. In

birds the B-lymphocytes develop in a lymphatic structure in the cloacal region known as the **Bursa of Fabricius**. It is still not clear what is the equivalent central lymphatic organ for B-lymphocytes in humans, though there is increasing belief that this is possibly also in the bone marrow.

PERIPHERAL LYMPHATIC ORGANS

Peripheral lymphatic organs include the **tonsils**, **lymph nodes** and **spleen**. The T- and B-lymphocytes migrate to peripheral lymphatic organs, where they populate specific regions. T-lymphocytes, for example, are located in the paracortical zone of lymph nodes, in the periarterial sheath of the white pulp of the spleen or in loose lymphatic tissue such as the Peyer's Patches. The immunocompetent T-lymphocytes are also the main lymphocytes found in the blood and involved in recirculation. B-lymphocytes are found in the various lymphatic tissues of the body (lymphatic nodules, loose and dense lymphatic tissue) apart from the sites of the T-lymphocyte populations.

Effector cells

In the peripheral lymphatic organs both T- and B-lymphocytes respond to exposure to antigen and enlarge to form **T-immunoblasts** or **B-immunoblasts** (or lymphoblasts).

(1) **Cytotoxic cell-mediated immune response**

The T-immunoblasts develop into **cytotoxic (cell-mediated) killer cells** (T-killer cells). These are the main cells involved in graft rejection. Some T-immunoblasts develop into **T-helper cells**, which cooperate with B-lymphocytes to stimulate their proliferation and differentiation.
Some T-lymphocytes develop into **T-memory cells**. These cells only become effector cells if the body is exposed at some future date to the same antigen. This is the basis on which vaccines work. There are also **suppressor T-cells**, that operate as inhibitory cells and play a role in determining that the duration of the immune response is limited. The suppressor T-cells depress antibody production by depressing the conversion of B-lymphocytes into plasma cells (i.e. antagonistic effect to T-helper cells).

(2) **Humoral-mediated immune response**.

The B-immunoblasts develop into **plasma cells** and secrete **antibodies**. There are also B-memory cells.

The cells circulating in the blood are mainly T-lymphocytes. T-lymphocytes and B-lymphocytes are morphologically identical, however they are distinguished on the basis of different surface markers e.g. T-lymphocytes bind to sheep erythrocytes, whereas B-lymphocytes do not. Today there are large numbers of surface markers to distinguish the various lymphocytes.

Natural Killer cells

There are cytotoxic lymphocytes that are independent of the thymus. These are present even in animals lacking a thymus ("nude" mice). These cells are immunocompetent and respond to foreign cells (tumor cells, virus-infected cells) by developing into **LAK cells** (lymphokine activated killer cells).

Summary of components of immune system

1. **Stem cells** (bone marrow) - source of lymphocytes

2. **Central Lymphatic Organs** (Thymus, Bursa analog) - site of antigen independent proliferation and differentiation to T- and B-lymphocytes

3. **Peripheral Lymphatic Organs** (Lymphocyte proliferation and exposure to antigens)

4. **Effector Cells** (T- and B- lymphocytes involved in immune responses)

5. **Other cells** (Macrophages, Dendritic antigen-presenting cells, Eosinophils, Memory Cells).

Antigen-presenting cells of the body

Antigen-presenting cells hold or trap antigens on their surfaces so that these can be presented to B-lymphocytes (effector cells) resulting in antigen-antibody complexes. Antigen-presenting cells of the body include:

(1) **Dendritic cells of lymphatic follicles**

(2) **Langerhans cells** of the epidermis

(3) Some **macrophages**.

Follicular dendritic cells are able to capture antigens on the surface of their processes for several months, allowing interaction with the surrounding B-

lymphocytes. The follicles represent a specialized microenvironment for the creation of memory B cells.

TONSILS

Tonsils are aggregates of lymphatic tissue, partly encapsulated, in the connective tissue associated with the oral and pharyngeal tract. They are best seen in the two **palatine tonsils** situated at the rear of the oral cavity and the start of the pharynx.

Stratified squamous epithelium with invaginations (**crypts**) covers the oral side of the tonsil. **Lymphatic nodules** and **dense lymphatic tissue** are present in the underlying lamina propria. The inner surface of the tonsils has a dense connective tissue capsule. Adjacent salivary glands are also commonly seen in histological preparations of tonsils.

LYMPH NODES

Lymph nodes are kidney-shaped structures situated along lymphatic vessels and serve as in-line mechanical filters of lymph. They are sometimes referred to as lymph glands, but this is a misnomer, as they are not glands and do not secrete.

The depression on the convex side of the node is called the **hilum**, which is the site of entry and exit of blood vessels and nerves. Lymph enters the node via a series of **afferent lymphatic vessels** on the convex surface and drains into a single **efferent lymphatic vessel** at the hilum. The lymph flow in the node is unidirectional, slow and passive. Valves in the lymphatic vessels prevent any backflow. A **capsule** of dense connective tissue surrounds the node. From this capsule extend radial **trabeculae** of connective tissue, to form incomplete compartments.

A **loose reticular tissue** extends throughout the node. This consists of reticulate cells and fibers, which form a network or scaffolding and which is well-demonstrated in preparations after silver impregnation techniques. This network provides an environment for the immunocompetent cells and antigen-presenting cells.

The lymph node is divided into two regions:

(1) An outer **cortical region**
(2) An inner **medullary region**

The **cortical region** consists of:

(a) **subtrabecular** and **peritrabecular sinuses**
(b) **lymphatic nodules**.

The **medullary region** is formed from branching **medullary cords** of lymphocytes, between which are found the **medullary sinuses**.

The **sinuses** of the node are irregular spaces formed of loose lymphatic tissue (reticular cells and fibers) containing various lymphocytes, antigen-presenting cells, macrophages. The sinuses act as mechanical filters in which lymph flow is extremely slow and are the sites where many cells are trapped. (This is the reason why biopsies of regional lymph nodes are examined to see if they contain cancer cells and the extent of the spread of the disease).

A **paracortical zone,** which lacks distinct morphological boundaries (situated between the cortex and the medulla,) is a region occupied by T-lymphocytes. This region has been shown to be **thymus-dependent** and if the thymus is removed experimentally, the paracortical zone disappears. Lymphocytes, mainly recirculating T-lymphocytes, leave the blood to repopulate the lymph nodes via **post-capillary venules**. These are blood vessels with unusual endothelium composed of tall or cuboidal endothelial cells. The T-lymphocytes can cross this endothelium to settle in the paracortical zone.

Antigens are trapped on the surface of antigen-presenting cells (**dendritic follicular cells**), where it is recognized and leads to B-cell activation to form B-immunoblasts and plasma cells, which produce specific antibodies. In cases of infection, lymph nodes become greatly enlarged.

THE SPLEEN

(1) The spleen weighs about 150g in adults and forms the **largest aggregation of lymphatic tissue in the body**.

(2) The spleen is the **largest lymphatic organ of the circulatory system** and acts as a **blood filter** to defend against microorganisms that succeeded to penetrate the blood system. Whereas lymph nodes are immunologic filters of lymph, the spleen is the immunologic filter of the blood.

(3) Many **aged erythrocytes are broken down** and destroyed by macrophages in the spleen. (Also a function of Kupffer cells in the liver).

(4) Like other peripheral lymphatic organs, the spleen is the site of formation of activated lymphocytes and responds promptly to antigens carried in the blood. Infection can result in an enlarged spleen (splenomegaly).

(5) About 30-40 ml blood are stored in the spleen. However, about 30% of all **blood platelets of the body are stored in the spleen** to meet physiological demands in cases of emergency. The spleen is easily ruptured in cases of traumatic injury (car accidents) and is commonly surgically removed to prevent continued hemorrhage.

(6) The **fetal spleen** (months 4-6) **is hematopoietic** (producing granulocytes and erythrocytes).

General structure

The spleen has a **dense connective tissue capsule**, with connective tissue **trabeculae** that divide the organ into incomplete compartments. The medial surface of the spleen has a **hilum**, which is the site for entry and exit of the blood vessels and nerves.

If a freshly cut spleen, small white spots (white pulp) consisting mainly of lymphatic nodules are visible embedded in soft, red, blood-rich tissue (red pulp).

White pulp

The white pulp consists of lymphatic tissue surrounding arteries and nodules also associated with arteries. The nodules can be distinguished because of the arteries (central arteries). Between the nodules and the red pulp is a marginal zone.

The lymphatic tissue immediately surrounding the central artery is known as the **periarterial lymphatic sheath** (**PALS**) and is composed of T-lymphocytes. The more peripheral part of the nodules is known as the **peripheral white pulp** (PWP) and consists of aggregates of B-lymphocytes. Dendritic cells in the marginal zone trap antigens and expose them to immunocompetent cells.

Red pulp

The red pulp is like a sponge composed of cords of cells (**splenic cords**) and **splenic sinusoids** (venous sinusoids).

The **splenic cords** (**Billroth cords**) are composed of:

(1) **reticular cells and fibers**
(2) fixed and wandering **macrophages**
(3) **lymphocytes**
(4) **plasma cells**

(5) **blood cells** (erythrocytes, granulocytes) and platelets.

The **splenic sinusoids** have dilated, irregular lumina. The endothelial cells are discontinuous, with very large gaps (2-3μm) between cells allowing easy passage for erythrocytes via the gaps. The basal lamina is also discontinuous. Reticular fibers form supporting hoops around the sinusoids. Macrophages can send their processes via the endothelial cells.

Blood circulation

(1) **Splenic artery** enters at the hilum and divides into trabecular arteries

(2) The **trabecular arteries** on leaving the trabeculae are completely surrounded by a sheath of T-lymphocytes (periarterial lymphatic sheath or PALS). The arteries are known as central arteries.

(3) The **central arteries** (or central arterioles) penetrate the lymphatic nodules, usually in the periphery.

(4) On exiting the nodules and entering the red pulp the vessels divide into several straight **penicillar arterioles**. These arterioles typically become surrounded with sheaths of macrophages (**ellipsoids**) before continuing as sinusoids.

(5) **Venous sinusoids** occupy the spaces between the splenic cords (Billroth cords).

(6) The sinusoids drain into **red pulp veins**, which lead to trabecular veins.

(7) The **trabecular veins**, located in the trabeculae, have no walls of their own, but only endothelium. These veins drain into the **splenic vein**.

There are two theories of circulation in the red pulp, which are not completely resolved. The "**closed circulation**" theory claims that the blood flows in a closed series of vessels. The "**open circulation**" theory claims that the blood from the penicillar arterioles freely enters all the spaces between the red pulp cord cells before draining into the sinusoids.

FINAL EXAMINATION

Final examination grades (%) are based on an examination that includes both theory and practical parts.

Students are required to succeed in both parts or they receive a fail grade. The time for the examination is 3 hours in total equally divided between theory and practical. The exam is closed (no supplementary materials are permitted in the examination room).

Theory examination (50%) consists of 40 Multiple Choice Questions, each with 5 true-false options and 10 drawings or photographs with 5 items to identify i.e. in total 250 questions.

Practical examination (50%) consists of 25 unseen histological preparations. Each student receives an individual set, in which the slide collections differ from set to set. The first 10 slides are based on tissues, each with 5 true-false answers. The remaining 15 preparations are based on specific identification of organs. Some preparations have more than one organ. Identification of areas of the digestive tract needs to be specific. Skin must be distinguished between thin and thick skin. Some of the slides include structures that are not in the strict sense "organs", such as large blood vessels, neurovascular bundles etc. The same organ may appear in more than one preparation.

No information is provided regarding the staining used, though most slides are stained with standard Hematoxylin and Eosin (H&E).

STUDENT EXAMINATION PERFORMANCE

Year	Average (%)	Failures*	# Students
1985	81	1	55
1986	77.7	7	78
1987	84.1	1	62
1988	82.4	1	61
1989	79.6	4	75
1990	82.3	1	67
1991	85	1	68
1992	83.5	0	64
1993	84.6	0	63
1994	No exam**	-	71
1995	79.4	8	78
1996	84.8	2	61
1997	78.8	7	65
1998	77.9	4	63
1999	78.6	2	63
2000	87.7	1	58

The figures represent results from only those students that took the examination on the official first date of the examination.

* Failures at first attempt (final grade less than 55%)

** Because of the prolonged academic strike of 1994 the examinations were cancelled, however students were given the option to sit the examination. 28 students out of 71 took the examination, with no failures.