THE ART OF EXAMINING AND INTERPRETING HISTOLOGIC PREPARATIONS: A LABORATORY MANUAL AND STUDY GUIDE FOR HISTOLOGY

Second Edition

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TABLE OF CONTENTS

INTRODUCTION

The examination and interpretation of tissue sections seen under the light microscope in a laboratory setting is an example of student-directed, independent problem solving. The proper reading of a histologic section is an acquired art that can only be developed through practice, close observation and repetition. This laboratory manual **was designed as a guide for students** to aid them in this endeavor. The laboratory study guide/manual was designed to be used as a supplement to any current textbook and/or atlas of Histology. **Learning objectives** provide the overall goals for each chapter. The narrative of the study guide explains how to systematically breakdown, examine and interpret each tissue and/or organ encountered, without regard to a given histologic slide from a specific slide collection. Thus, this systematic method can be used to examine and interpret histologic preparations from any collection or of any species.

The **student is encouraged to sketch, label and create a personalized atlas** while using this laboratory manual as a guide. The *vocabulary* that should be developed and used during the laboratory can be found quickly by going to the **bold face** type in the appropriate segment of the text. Each chapter contains one or more **tables** in which key structures used in the identification of a tissue/organ are presented, offering the briefest possible summary of important histologic features. As a final short review, an **appendix** provides summary tables that compare and contrasts the basic differences of several structures that are somewhat similar in general architecture.

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Have an appreciation of how a histologic preparation is made

The human body consists of two basic components: **cells** and **products of cells (extracellular materials)**. The discipline of histology is concerned primarily with the microscopic examination of these two components and how they are organized into the various tissues and organs of the body.

Obviously, if the liver, or a similar organ, were to be examined, it would be impractical to place the entire organ under a routine light microscope for study. It is not only much too large, but also opaque, therefore an examination of its microcomponents would be impossible. For this reason, and several others, a small portion of a specific tissue or organ must be **excised** from a given organ and processed for microscopic analysis. The excised tissue is placed, as soon as possible after removal, into a reagent known as a fixative. **Fixatives** act to preserve the cells and extracellular substances of tissues/organs and prevent autolytic (degenerative) changes. Although there are numerous fixatives developed for a variety of purposes, 10% buffered formalin is one of the most commonly used, routine fixatives in biology, medicine (surgical and general pathology) and biomedical research. The collected tissues, once fixed, are then **dehydrated** in graded solutions of alcohol or other dehydrating agents. Following removal of the majority of water from the collected specimens during dehydration, the tissues are cleared. **Clearing** is the process of removing the dehydrating agent and replacing it with a fluid that is miscible both with the dehydrating agent used and with the type of embedding medium chosen to make the tissue sample firm throughout. As with dehydrating agents, there are a large number of clearing reagents, the selection of which is dependent largely on the embedding medium chosen. Xylene and toluene are in common use for paraffin embedding; propylene oxide for embedding in several of the plastic embedding media. The tissue sample is next **infiltrated with** and **embedded in** the chosen embedding medium so that a firm homogeneous mass of material containing the tissue sample is obtained. Paraffin is the most commonly used embedding medium for routine preparations. The formed paraffin block, together with the contained tissue, is then sectioned (cut) into very thin slices called **sections** that normally range between 4 and 7 microns (μm) in thickness. The instrument used in cutting histologic sections is called a **microtome**. The cut sections are then transferred (mounted) onto the surface of clean glass microscope slides.

In order to prepare the **mounted sections** for staining, the paraffin embedding medium must be removed. This is accomplished by passing the slides together with their mounted sections through xylene or toluene to remove the paraffin and then through descending strengths of alcohol solutions to water, as most dyes used are in aqueous solutions. **Staining** is a process of increasing the visibility of cells by the application of dyes or by the reaction of chemical reagents with the tissue components to form visible substances. A large number of stains are available but generally only two stains are used together to provide contrasting color: one to stain the cytoplasm of cells, the other to stain the nuclei. The most common and universally used combination is the **hematoxylin and eosin (H&E) stain**. When this stain is applied to a section of tissue the **nuclei** of component cells appear **blue**; the **cytoplasm** and most **extracellular materials** a light **pink-orange**. Staining is necessary because the vast majority of cells and their extracellular materials are transparent and lack color. Only naturally occurring pigment granules such as melanin and lipofuscin would be visible on examination. The color of the dyes used during staining markedly increases the contrast of cells, their sub-components, and the associated extracellular materials. Without staining, the examination of cells and tissues with a routine light microscope would be extremely difficult. Following staining in aqueous dyes, the slides, together with their mounted, stained sections, are passed back through ascending concentrations of alcohol for dehydration, cleared with some solvent (usually xylene or toluene), and then a permanent mounting medium is put on the tissue section. A thin glass cover slip is then placed on the covering mounting medium and underlying tissue section and allowed to dry. As the histological preparation dries, the solvent evaporates from the mounting medium, which hardens, permanently cementing and sealing the tissue preparation between the glass slide and overlying cover slip. The mounting medium (balsam, damar, Permount) when dried has nearly the same refractive index as glass. After drying, the histologic section is well protected and if stored properly will usually remain unchanged for several years.

Know what you are looking for

Before examining a histologic preparation of any given tissue or organ, know something of the structure before studying it under the microscope. Such familiarity is usually acquired by carefully considering the details of the tissue/organ in question and formulating a mental image of how the structure should appear. The microscope should then be used to confirm or refute the preconceived image conceptualized. Examination of tissues and organs without prior thought and consideration of the subject usually proves frustrating and is often a waste of considerable time. Therefore, before attempting to examine any specimen for the first time with the microscope, know as much about the structure of the subject matter as possible. This information can only be acquired by attending lectures and/or reading textbooks and studying atlases.

It is **highly recommended that a small labeled sketch be made** of each section examined under the microscope using colored pencils, noting relationships and the position of specific structures and cells. This simple exercise aids in focusing concentration on the structure(s) being examined and avoids casual observation. The construction of such a labeled, personalized atlas aids in cementing the observations made in one's memory and is important in beginning to develop a mental three-dimensional image (understanding) of the tissues/organs examined from the two-dimensional image presented by the histologic preparation. The labeled sketch also serves as a highly personalized map of a specific slide for re-examination later in the exercise and is excellent for review purposes.

Making sketches

A variety of sketches should be used dependent on the structural detail needed to clearly understand a given topic. Use of several different types of sketches is suggested, the one chose dependent on the needs of the exercise.

For example: in the first exercise recommended (the identification of large cells), a simple line sketch of the entire tissue section can be used with a label showing precisely which region was used to look for and examine cells. In the case of the spinal cord, a simple outline of the tissue is all that is needed, in which nerve cells (neurons) should be identified. A supplemental sketch depicting and labeling the salient points of a neuron (cell shape, cytoplasm, position and shape of the nucleus, nucleolus and nuclear envelope) should also be used.

The other example suggested for this very important initial exercise is the identification of another of the extraordinarily large cells (ova) in the ovary. Take care to note their position in this organ and then examine the surrounding tissue for additional cells that will show a variety of different sizes and shapes. In this case, a more detailed sketch should be used to illustrate the latter points as well as focusing on the **most important aspect of the exercise:** being able to distinguish clearly between the nucleus and the cytoplasm of a given cell and to estimate nuclear and cell boundaries.

Later, sketches can be used to illustrate how cells are organized into units and how understanding such organization has led to various classification schemes, as with the classification of epithelial tissues. In this case, greater detail should be employed to illustrate cell shape, size and organization. Further details, such as modification of the cell membrane (plasmalemma) or attachment points, should also be noted.

When the histologic makeup of entire organs is considered, sketches with less cellular detail are often useful as guides as to where a particular type of tissue is located in a given organ. This is particularly true of tubular structures, the walls of which are formed by several different layers or strata. Additional accompanying detailed sketches may be needed if an important characteristic group of cells or other structures are present within the organ.

Reliable mechanics on how to examine histologic preparations (slides)

When presented with a histologic preparation (slide), the very first exercise that needs to be done is to **examine it closely with the naked eye**. A considerable amount of important information can be ascertained about the preparation using this simple exercise **before placing the slide under the microscope**. Indeed, this exercise is of such importance it should become part of the "*standard operating procedure*" for each slide examined. Initially pick up the histologic slide between the thumb and index finger and examine it by holding it up to the light or against a white background. **What to look for:** First, look at the overall nature of the preparation. Does the preparation have a doughnut configuration? If so, this immediately suggests to the viewer that the specimen being examined is tubular in nature and is being viewed in a transverse profile (hollow organs, such as blood vessels, regions of the digestive tube, tubular components of respiratory, urinary and reproductive systems, are possible organs and this list can be restricted even further if dealing only with human tissues by the size of the preparation.

Identification of the luminal surface (the lining of the internal space) as well as the external surface will be of importance when this preparation is examined further. Does the preparation have a uniform consistency and appear as a solid mass of tissue cut in the shape of a square, rectangle or wedge? If so, such a preparation usually indicates that the sample of tissue was taken from a large compact (solid) organ such as the liver, spleen, kidney or pancreas to list just a few. Once a determination has been made with regard to the shape and consistency of the tissue mounted on the slide, then examine it more carefully with regard to its staining characteristics. Of particular importance is to note if one surface (external edge or luminal surface of the doughnut-shaped configuration) stains more basophilic (light blue) than any other region in the sample of tissue being examined. Such basophilic staining usually indicates a high concentration of nuclei per unit area (nuclei stain blue with hematoxylin dye). In this way, one of the basic tissues, epithelium, can usually be located even before the histologic slide is ever viewed under the microscope. Are additional small tubular or round structures present within the tissue sample? These may indicate small blood vessels, ducts or glandular structures.

Always begin the initial examination of a histologic preparation **with the low-power (scanning) objective** and carefully **view the entire section**. This opportunity should be used to confirm or deny the observations and speculations made by direct observation with the naked eye. Add more details to the mental image being developed with regard to the preparation under examination. Note the presence or absence of more than one tissue type, patches of deeper staining, other structures present, their locations and relationships to one another and to surfaces. Only after a thorough examination with the low-power objective should the intermediate- and high-power objectives be used. Of these, the medium-power objective is the more useful for study, although more detail can be seen with the high-power objective. The disadvantage of the high-power objective is the smaller field of view and, because of this, relationships between tissues are often lost. The oil objective, if used at all, should be used only in the examination of peripheral blood and bone marrow preparations.

Since the tissues and organs of the body consist only of two elements, cells and cell products (extracellular materials) both deserve careful and thorough study. The initial exercise should be to examine the **morphology of a cell**. The following observations should be made during the examination of various cell types.

1. Shape of the cell.
2. Size of the cell (determine the position of the cell membrane).
3. Shape, size and position of the nucleus.
4. Identify the nucleolus if present in the nucleus.

It is absolutely essential that the boundary of the cell and that of the nucleus be clearly defined. Examine several cell types of various sizes and shapes to make these observations.

Large cells

Examine a section of ovary for ova. These are located in the ovarian cortex at the periphery of the ovary. The ovum represents a very large, round cell. Because of their large size, these light-staining cells can be found, by using the low-power objective, near the periphery of the ovary. After examining an ovum at low power, study it further using increased magnification. Note again the large size of the ovum, the abundant light-staining cytoplasm, and the central round nucleus separated from the cytoplasm by a well-defined nuclear membrane (envelope). Identify the **nucleolus**. It is usually round in profile and stains intensely. The nuclei of several ova may have to be examined, as these cells are so large that the plane of section may not pass through the nuclear region containing the nucleolus in all ova. Examine the remainder of the ovary and note the differences in the size and shape of the different cell types. Note that the nuclear shapes most often assume the shape of the cells being examined and that the cell membrane of most cells cannot be resolved with the light microscope. Therefore, when examining a number of tissues and organs the nuclei of component cells are often relied on in determining the orientation and shape of the cellular component and the cytoplasmic boundaries of a give cell type are estimated. The shape of the ovum is generally spherical, that of surrounding cells cube-shaped, whereas more distant cells in the ovary are spindle in shape. Examine the nuclear profile of each group of cells, noting how the shape of the nucleus reflects the shape of the cell. In addition, note the dark-staining, clumped nature of the chromatin is some nuclei. This material is referred to as **heterochromatin** and often lies adjacent to the nuclear envelope. The lighter-staining nuclear material is referred to as **euchromatin**.

The next histologic preparation that should be examined at this time is a transverse section through the spinal cord. Visual examination of the preparation will reveal an **H**-shaped area (gray matter) near the center of the preparation that surrounds a small central canal.

Examine the gray matter with the low-power objective and locate numerous, large neurons (nerve cells) found in the ventral horn. These too are exceptionally large cells with several irregularly shaped, elongated processes. Make a clear distinction between the cytoplasm, nucleus and nucleolus.

Make a small labeled sketch of several cells from these preparations illustrating the cytoplasm, the size and shape of the nucleus and the position of the nucleolus.

Cytology: structural components of a cell that can be examined with the light microscope

This laboratory guide briefly presents **a method for examining the cytologic and histologic details of human morphology** utilizing the routine H&E preparation. This preparation primarily demonstrates the nucleus and the surrounding cytoplasm of a given cell. It must be understood, however, that special staining methods can be used to demonstrate the majority of organelles, inclusions and components of the cytoskeleton within a given cell, as well as a variety of cell products and extracellular materials. These special staining methods include a variety of dyes, antibodies and other probes.

Techniques such as **immunohistochemistry**, ***in situ* hybridization** and **autoradiography** are powerful tools in demonstrating structure, secretory products, and/or messages (mRNA) **within** cells, as well as cell receptors not seen with routine preparations. The study guide focuses primarily on what can be visualized using the routine H&E preparation unless otherwise stated.

Know the basics: the basic tissues

The term **tissue** [French tissu, woven cloth] is defined as a collection of similar cells and surrounding extracellular substances that perform related functions. **Four basic tissue types** occur and these are woven together to form the fabric of all organs.

The four basic tissues are: **epithelium**, **connective tissue**, **muscle tissue** and **nervous tissue**. A thorough understanding of each of these four basic tissues is necessary before beginning an examination of individual organs or systems.

CHAPTER 2. EPITHELIUM

Epithelia consist of closely aggregated cells separated by only minimal amounts of intervening intercellular substances. Two general categories are recognized: ***a lining, barrier or covering type of epithelium*** organized into sheets of cells that form barriers and ***glandular epithelium*** modified for secretion. The sheet (barrier) form of epithelium covers the external body surface as the epidermis, lines the body cavities (pleural, pericardial, peritoneal) as well as the lumina of the cardiovascular, digestive, respiratory and urogenital systems. Thus, the majority of, if not all, substances entering or exiting the "substance of the body" must first cross an epithelial barrier. All epithelia lie on a basement membrane and are avascular. The **basement membrane** appears as a thin interphase between the epithelium and underlying connective tissue. It consists of glycoproteins, proteoglycans rich in heparin sulfate and type IV collagen. Usually the basement membrane appears only as an interphase in H&E preparations but can be demonstrated clearly using special staining techniques. As different types of epithelium are examined, a mental record should be established, keeping track of not only where a specific type of epithelium is found in a given organ but also its functional ramifications. Later, in the examination and identification of organs under the microscope, the identity and location of a specific **epithelium** is often a **key feature** in the identification.

Lining/covering (barrier) form of epithelium

Learning objectives for lining or covering epithelium:

1. Be able to locate, identify and classify the various types of epithelia in a given section of histologic material.
2. Be able to identify the various specializations of the cell membrane associated with specific epithelia.

Classification

If the covering or lining form of epithelium consists only of a single layer of cells it is termed **simple.** If two or more layers of cells are present, of which the superficial most-cells do not reach the basement membrane, the epithelium is classified as **stratified.** Once this determination has been made the next step is to determine the **geometric shape** of the **superficial-most cells** to complete the classification. Epithelial cells can be divided into three types according to their geometric shape: **squamous** (thin, flat, plate-like cells), **cuboidal** (height and width of the cell are approximately equal with the nucleus nearly touching all surfaces), and **columnar** (height of cell is greater than its width). Cells intermediate in height between cuboidal and columnar also occur and are referred to as low columnar. Thus, epithelium consisting of a single layer of cells can be classified as: simple squamous, simple cuboidal, or simple columnar. A fourth type of simple epithelium, **pseudostratified columnar**, consists of more than one cell type whose nuclei occur at different levels falsely suggesting that the epithelium is made up of two or more layers. All cells of this type of epithelium reach the basement membrane but not all reach the luminal surface.

The term **endothelium** is the specific name given to the simple squamous epithelium that lines the cardiovascular and lymph vascular systems. Examine the luminal (interior) surface of several blood vessels for this type of epithelium. The name **mesothelium** is given to the simple squamous epithelium that lines the pleural, pericardial and peritoneal cavities. Examine the external surface of a region of the stomach, jejunum or ileum for mesothelium. Examine the luminal surface of each of these organs for simple columnar epithelium. **Note:** Prior to examining these organs under the microscope, each should be examined with the naked eye. As an epithelium must lie on one surface or the other, and in the case of the gut, both, examine each of these surfaces under the microscope initially with low power for orientation prior to moving to higher power objectives for a more detailed examination.

Simple squamous epithelium

Examine the external surface of the stomach or small intestine. Note that the cells making up this form of simple squamous epithelium appear extremely attenuated, with their flattened, dense nuclei separated by considerable distances. The cytoplasm often appears only as a thin interphase between nuclei.

Simple columnar epithelium

Examine the luminal surface of the stomach for typical simple columnar epithelium. Note the basal position of the oval-shaped nuclei and that the apical cytoplasm is filled with unstained secretory granules. These can be visualized more clearly by lowering the intensity of the light and/or by dropping the condenser of the microscope. **Terminal bars** also can be seen in most preparations between the apices of adjacent cells.

They appear as minute, dense-staining short bars and represent the light microscopic appearance of the three part junctional complex (zonula occludens, zonula adherens, macula adherens) seen with the electron microscope. Examine a textbook illustration (electron micrograph) of this important junctional specialization. Examine the luminal surface of a region of small intestine. Note that it, like the stomach, is lined by a simple columnar epithelium. Carefully examine the intestinal lining epithelium and identify the **striated (microvillus) border** on the apical surface. Terminal bars also may be seen between the apices of cells forming this epithelium, as well as most other simple columnar or cuboidal epithelia.

Simple cuboidal epithelium

This type of epithelium is best seen in a section of kidney medulla, which also contains numerous tubules lined by either a simple columnar or simple squamous epithelium. Tubules lined by either simple squamous, simple columnar or simple cuboidal should be identified and compared. The nuclei of cells in a good simple cuboidal epithelium should nearly touch apical, basal and lateral cell membranes. As different tubules are examined, several examples of "low columnar" will also be encountered.

Sketch and compare tubules formed by classic examples of the three types of simple epithelia observed.

Stratified squamous epithelium

Stratified squamous is the most common of the stratified epithelia. A good example of stratified squamous is the epidermis of skin. Once again, examine the slide visually, noting the surface on which the epithelium rests, then examine it under low power. Note the presence of additional components of the skin but do not examine them at this time. Select an area of epidermis and examine it carefully under increased magnification, beginning at the basal surface. It should be quite obvious that this form of epithelium is multilayered and consists of cells that vary in their geometric shape. Cells resting on the basement membrane are columnar in shape whereas those above assume a more spindle-shaped configuration. In the outermost (superficial) layers, the cells become flat and plate-like, ie. squamous. Despite the large number of cells with different shapes, recall that the classification scheme remains based on two questions:

1. Are two or more layers of cells present, the outermost of which is not in contact with the basement membrane?

2. What is the geometric shape of the superficial-most cells?

Thus, the classification must be stratified squamous. In the case of the epidermis the superficial most cells also undergo a transformation, known as **keratinization**. As a result of this process, cells loose their nuclei and the cytoplasm becomes filled with a proteinaceous material called **keratin**. These transformed dead cells lie immediately above the intact layer of squamous cells. When this type of surface feature is present, it is usually incorporated into the terminology of the classification scheme of the epithelium. Thus, in the case of the epidermis, the complete classification of the epithelium would be **keratinized stratified squamous epithelium**. Repeat this exercise examining the epithelium lining the lumen of the esophagus. This epithelium lacks the layer of keratin on its luminal surface. Therefore, it is classified as a **non-keratinized (wet) stratified squamous epithelium**. The term wet is often used when this type of epithelium makes up a portion of a mucous membrane or lines a moist environment. Note the presence of intact nuclei in cells comprising the superficial most layer of this form of stratified squamous epithelium.

Compare the overall thickness of this epithelium with that of the epidermis and make a labeled sketch of each.

Stratified cuboidal

This type of epithelium is limited in distribution to regions of ducts from larger glands where there is a transition from a simple epithelium into a stratified epithelium. Stratified cuboidal epithelium also lines the ducts of sweat glands. Sweat glands are coiled tubes comprised of epithelial cells that extend from the base of the epidermis into the tissue of the underlying dermis and hypodermis. Because of their coiled nature, a profile of an entire sweat gland is rarely if ever encountered in sectioned material. Using the low-power objective, scan the tissue beneath the epidermis in a section of skin looking for groups of small circular and oval profiles. These cellular profiles are often encountered surprisingly deep within the underlying tissue. Two profiles will be encountered: one light staining, the other subtlety darker staining. The more darkly stained portion of the tubule is the duct region of the sweat gland. Confirm that the duct region consists of a stratified cuboidal epithelium, two cells thick, which surrounds a minute central lumen.

Sketch the duct region of a sweat gland to illustrate a stratified cuboidal epithelium.

Stratified columnar

Like stratified cuboidal, stratified columnar is restricted in its distribution confined primarily to the cavernous urethra, fornix of the conjunctiva and large excretory ducts of major glands. This epithelium is most conveniently observed in the large ducts of the parotid or submandibular gland. Examine one of these glands for the ducts only. They can be identified initially with the low-power objective by scanning the gland and looking for tubules with a very large luminal diameter. Once located, examine the wall of the duct and determine the nature of the lining epithelium. Is it stratified? Is the epithelium stratified cuboidal or stratified columnar? Both types will be encountered. Search the preparation until an example of each is found.

Make sketches of two large ducts: one lined by stratified cuboidal, the other lined by stratified columnar.

Pseudostratified columnar

This type of epithelium is a simple form of epithelium as all component cells are in contact with the underlying basement membrane, thereby satisfying the definition of a simple epithelium. The cells vary considerably in height and not all reach the luminal surface. As a result, the respective nuclei are found at different levels within this epithelium and form what appears to be two or three layers of cells, falsely suggesting stratification, hence its name. Pseudostratified columnar epithelium is somewhat restricted in its distribution, being confined primarily, but not exclusively, to the conducting portion of the respiratory system and the excurrent ducts of the male reproductive system.

Examine the trachea and epididymis for examples of this type of epithelium. Examine the luminal surface of the trachea and note the height of the epithelium and the stratified appearance due to the position of component nuclei. With the high-power objective examine the epithelium in detail beginning at the base noting the different sizes and shapes of cells forming this epithelium. Scattered within this epithelial layer are unicellular exocrine glands known as goblet cells. **Goblet cells** are sandwiched among the other epithelial cells and usually have a drinking goblet (wineglass) shape. The base of this cell is usually very narrow and contains the nucleus. The apical region is expanded due to the presence of numerous mucin granules. The latter are unstained in H&E preparations and appear as clear vacuoles. With special mucin stains they appear solid and stain brilliantly. Examine the apical surface of the pseudostratified columnar epithelium for apical specializations called cilia. **Cilia** appear as small tufts or hair-like structures protruding from the apical cell surface. When cilia are encountered they are usually included as a prefix in the name of the epithelium. Hence, the epithelium lining the trachea would be termed a ***ciliated pseudostratified columnar epithelium***.

Examination of the epididymis with the low-power objective reveals an organ that consists of several profiles of small tubules. In actual fact, the epididymis consists of one extensively coiled, elongated tubule. An appreciation of this fact at the start is of importance in developing three-dimensional mental reconstructions of images from two-dimensional images examined under the microscope. Examine the pseudostratified columnar epithelium lining the epididymal tubule at increased magnification. Note that it consists of two distinct cell types: a small basal cell and a tall columnar cell known as the principal cell. The latter have elongate, branched microvilli extending from their apical surface, which are called **stereocilia**.

Make a sketch of the ciliated pseudostratified columnar epithelium lining the trachea and compare it with an additional sketch illustrating the pseudostratified columnar epithelium lining the ductus epididymidis.

Transitional epithelium

Transitional epithelium is a stratified cuboidal type of epithelium found only in the urinary system. Examine this epithelium lining the interior of either the urinary bladder or ureter. Note the thickness of this epithelium and the large dome-shaped cells at the luminal surface. The latter may be binucleate.

Make a sketch of transitional epithelium from the lining of the ureter.

Specializations associated with the cell membrane of epithelial cells

Microvilli

Closely re-examine the apices of cells forming the simple columnar epithelium lining the small intestine. Note the **striated (microvillus) border** found at this location. Next, find and examine the proximal convoluted tubule of the kidney. This tubule is found only in the renal cortex (the outer region of the kidney), is the longest tubule in the cortex (therefore exhibits the most numerous tubular profiles) and is the most granular and darkly stained tubule. The apices of cells forming the proximal tubule exhibit numerous microvilli closely packed together to form the **brush border** of light microscopy.

Re-examine the **stereocilia** on the apices of the epithelial cells lining the tubule of the epididymis. Stereocilia are a thin, highly branched form of microvillus.

Cilia

Re-examine the ciliated pseudostratified columnar epithelium lining the trachea. Note that individual cilia can be seen. If the preparation is good (particularly if the embedding medium is a plastic resin of some type) numerous **basal bodies** can be visualized in the apical cytoplasm immediately beneath the cilia and appear as a dense beaded line. Examine the ciliated simple columnar epithelium lining the oviduct for the same features.

Make sketches comparing the apical specializations found on the epithelial surfaces examined above. In addition, examine several textbook illustrations of each apical specialization for their ultrastructural features.

Basal striations

Two organs that contain cells that show excellent examples of this specialization of the cell membrane are the distal convoluted tubules of the kidney cortex and the striated ducts of the submandibular gland. Re-examine the cortical region of the kidney with the low-power objective for light-staining tubules. When examined at higher magnification note that these lighter-staining epithelial cells show distinct nuclear profiles and lack the microvillus border observed in cells forming the proximal convoluted tubules.

Careful examination of the basal cytoplasm of cells forming the distal tubule will reveal faint striations. The intensity of light in the microscope may have to be decreased and/or the condenser lowered to make the basal striations more visible. With special staining techniques (iron hematoxylin - which actually demonstrates mitochondria) the basal striations are dramatic. Basal striations represent a complex infolding of the basolateral cell membrane plus a parallel arrangement of associated mitochondria. Examine a section of submandibular gland for a smaller caliber duct within the lobules of the gland. The striated ducts are intralobular ducts and appear as numerous circular profiles with wide lumina within the glandular tissue. The epithelium lining these ducts is simple cuboidal to columnar in type, is light staining and agranular. Close observation of the basal cytoplasm using the same conditions as when examining the distal convoluted tubule of the kidney will demonstrate faint basal striations within cells making up the striated ducts. **Note**: these ducts were so named because of the basal striations. Examine a textbook electron micrograph of cells from the distal convoluted tubule of the kidney for basolateral infoldings. Compare these features with those seen with the light microscope.

Make a sketch illustrating this morphological feature.

Table 1. Location of epithelia.

Type of Epithelium	Location	Specialization
Simple squamous	Endothelium, mesothelium, thin segment of loop of Henle, rete testis, pulmonary alveoli, parietal layer of Bowman's capsule	
Simple cuboidal	Thyroid, choroid plexus, ducts of many glands, lens epithelial cells, covering surface of ovary, corneal endothelium	
Simple columnar	Surface lining epithelium of stomach, gallbladder, ducts of several glands	
	Surface lining epithelium of small and large intestines	Striated border
	Proximal convoluted tubule of kidney	Brush border
	Distal convoluted tubule of kidney	Basal striations
	Oviducts, uterus, small bronchi and bronchioles	Cilia
Pseudostratified columnar	Trachea, major bronchi, eustachian tube	Cilia
	Large excretory ducts of glands, portions of male urethra	
	Epididymis	Stereocilia
Stratified squamous	Esophagus, epiglottis, corneal epithelium, vagina	
	Epidermis of skin	Keratin
Stratified cuboidal	Ducts of sweat glands, large ducts of salivary glands	
Stratified columnar	Large ducts of glands, cavernous urethra	
Transitional	Restricted to urinary system: renal calyces to urethra	

Glandular forms of epithelium

Glands are comprised of epithelial cells specialized to synthesize and secrete a product of some type. A variety of different criteria can be used in the classification of glands. The simplest classification scheme is to divide the glands into **endocrine** (secretion into the lymph/vascular system) and **exocrine** (secretion onto an epithelial surface or into a duct) glands. A consideration of the endocrine glands will be presented later as a specific topic.

Exocrine glands can be classified further as to whether they are unicellular or multicellular. A classic example of a **unicellular exocrine gland** is the goblet cell. Re-examine the epithelium lining the intestine and trachea for goblet cells. Carefully study this cell, paying particular attention to its overall shape, position of the nucleus, and the apical accumulation of secretory (mucin) granules. Recall that the mucin granules, although unstained with the H&E preparation, can usually be visualized by lowering the intensity of the light and by lowering the condenser.

The majority of glands are **multicellular exocrine glands**. Multicellular glands can assume a wide variety of morphologies. They may occur as a small group of secretory cells that lie wholly within an epithelial layer, clustered about a small lumen. These are called **intraepithelial glands**. An example of this glandular organization can be found in the non-keratinized stratified squamous epithelium lining the penile urethrae of the male reproductive system. Examine this lining epithelium. The intraepithelial glands (glands of Littré) appear as clusters of clear or light-staining cells within the more darkly stained lining epithelium. The nuclei of component secretory cells are generally compressed to the base and the apical portion of the cell is filled with unstained mucin secretory granules. A somewhat similar glandular organization is the **secretory sheet**. In this case, the cells form a continuous epithelial layer. An example of this type of multicellular gland is the gastric lining epithelium of the stomach. This glandular form consists of a simple columnar, mucous secreting epithelium that secretes directly into the lumen of the stomach. Examine this epithelial form again.

The majority of multicellular, exocrine glands secrete into a ductal system. These are classified according to the morphology of the ducts and how their secretory cells are arranged to form the secretory portion of the gland. If the **duct branches** the gland is classified as **compound**; if the **duct does not branch** the gland is classified as **simple**. The secretory cells of the gland may be arranged into **tubules** and/or **acini** (**alveoli)** (berry-like end pieces). Subsequent classification depends on the shape and configuration of the secretory unit and whether these portions also branch. Thus, **simple glands** can be classed as simple tubular, simple coiled tubular, simple branched tubular, or simple branched acinar (alveolar). **Compound glands** are subdivided into compound tubular, compound acinar and compound tubuloacinar (compound tubuloalveolar).

Learning objectives for glandular epithelia and exocrine glands:

1. Be able to classify glands according to their histologic organization, type of material secreted and manner in which material is secreted.

Simple glands

Simple tubular glands

Examine a section of colon with the low-power objective. Find and examine the luminal surface of this organ and note that it is lined by a simple columnar epithelium. Observe the large number of goblet cells. Identify tubular invaginations that extend from this epithelium into the underlying tissue. These are the simple tubular glands (intestinal glands) of the colon. The epithelium forming the walls of these glands is the same as that lining the surface. If the plane of section through the wall of the colon is at an oblique angle, the glands may appear as isolated oval or circular collections of columnar cells surrounding a tiny lumen. If the glands are cut parallel to their long axis, the lumen of the gland can be traced to that of the colon. Next, examine a section of small intestine. In the small intestine, the simple tubular intestinal glands open between the bases of fingerlike extensions of tissue covered by simple columnar (intestinal) epithelium called villi. Compare the glands at this location with those of colon. Note the numerous mitotic figures within the epithelium forming these glands.

Draw and label a sketch of these structures, including the location of the simple tubular intestinal glands in a section of colon and small intestine.

Simple coiled tubular glands

Re-examine the section of skin for eccrine sweat glands as an example of a simple coiled tubular gland. Using the low power objective examine the deep subcutaneous tissue far beneath the overlying keratinized stratified squamous epithelium for these glands. The duct is long, extending from the surface epithelium to deep within the underlying tissue.

The secretory portion ends as a highly coiled structure similar to that of a coiled snail shell. A section through such a unit results in several circular cross-sectional profiles. These are lined by lightly stained simple columnar epithelial cells. The ducts are more darkly stained and lined by stratified cuboidal epithelium.

Simple branched alveolar glands

Continue to examine the preparation of skin for sebaceous glands. These glands are classified as simple branched alveolar glands and are almost always associated with hair follicles. The latter are invaginations of the epithelium into underlying tissue that produce and contain hair shafts. Study the sebaceous glands carefully. Note the presence of large flask-shaped secretory units, the alveoli. Alveoli of sebaceous glands consist of large cells filled with small lipid droplets, which gives them a light-staining, vacuolated appearance. Note that two or three alveoli drain into a single, short unbranched duct lined by stratified squamous epithelium. The duct empties into the lumen of an adjacent hair follicle. The overall three-dimensional shape of these glands is similar to a three or four leaf clover.

Sketch, label and compare a simple coiled tubular sweat gland with a simple branched alveolar (sebaceous) gland of the skin (integument).

Simple branched tubular glands

Study a section taken from the pyloric region of the stomach. Identify the gastric pits. These are tubular invaginations of the gastric surface lined by simple columnar epithelium that extend into the underlying tissue. Emptying into the bottoms of the gastric pits are the pyloric glands, an example of simple branched tubular glands. Cells forming these glands have basal nuclei and a light/clear supranuclear cytoplasm that contains mucin granules. Note that in this glandular form, the duct remains unbranched (simple) and that it is the secretory tubule that branches.

Sketch and label the subcomponents of a simple branched tubular gland from the pyloric region of the stomach.

Compound glands

Compound tubular glands

Examine a slide of the duodenum and scan it carefully. Find the location of numerous light-staining, secretory tubules of the duodenal (Brunner's) glands within the intestinal wall. These glands are found in the tissue beneath the bottoms of the simple tubular intestinal glands examined earlier in other regions of the intestinal tract. Now examine the duodenal glands at increased magnification. The terminal portions (secretory units) of the duodenal glands are branched, coiled and of uniform diameter. Component cells are light staining with basally positioned nuclei. The branching ducts are lined by a similar appearing epithelium to that forming the secretory units. The ducts of the duodenal glands unite with the bottoms of the overlying intestinal glands. Note the differences in the lining epithelium between glands where this transition occurs.

Sketch and label a duodenal gland including its association with an overlying simple tubular intestinal gland.

Compound tubuloacinar (alveolar) glands

This type of gland represents the most common glandular organization of the compound glands. As an example, study a section of the submandibular (submaxillary) gland with the low-power objective identifying several important features. The gland is organized into lobes and lobules. These glandular subdivisions are limited by fibers of the surrounding connective tissue. Next identify the duct system. The ducts can be recognized by their round profiles, wide lumina, and the light-staining cytoplasm of component cells. The epithelial lining is usually simple cuboidal or simple columnar although stratified forms of both types can be found on occasion lining the very large ducts. Note that two categories of ducts can be recognized: intralobular ducts and interlobular ducts. The former occurs **within** the lobules and in the submandibular gland are the most numerous. The interlobular ducts are larger and occur **between** lobules. The lining epithelium is usually simple cuboidal or simple columnar. Can basal striations be observed with increased magnification? Because the branched ductal system of the submandibular is well developed, numerous profiles of the ductal system are observed. Carefully examine the apices of cells forming the ductal epithelium. Note the minute, dense staining points between cell apices. These are terminal bars. Next, examine the secretory units of the submandibular gland and observe that two markedly different cell types makeup the tubules and acini (alveoli) of this gland. The most numerous are **serous cells**. Serous cells are characterized by a dark staining cytoplasm filled with numerous, distinct secretory granules. The basally positioned nuclei usually exhibit a round or oval profile. The less numerous cell type making up scattered tubules is the **mucous cell**. These cells are characterized by a white (clear) cytoplasm of frothy appearance. The latter is filled with unstained mucin granules.

Nuclei appear as darkly stained, compressed profiles positioned adjacent to the basal cell membrane. Some serous cells are organized into small units called **demilunes** that cap terminal regions of the scattered mucous tubules.

When both serous and mucous cell types make up the secretory units of a gland, the gland is often referred to as a **mixed gland**.

Sketch and label the subcomponents of a lobe from the submandibular gland.

It is important to realize from the onset that the connective tissues are classified according to the ***type*** and ***arrangement*** of the ***extracellular materials*** rather than features of the cellular components, as is true of epithelium. General connective tissues are classified as loose or dense according to whether the extracellular materials are loosely or tightly packed. **Loose** connective tissue can be subdivided further on the basis of special constituents such as adipose (fatty) tissue or a concentration of specific extracellular fibers. **Dense** connective tissue can be subdivided according to whether the extracellular fibers are randomly distributed (dense irregular connective tissue) or orderly arranged (dense regular connective tissue).

Loose (areolar) connective tissue

Areolar connective tissue is a loosely arranged connective tissue that is widely distributed throughout the body. It consists of three extracellular fibers (collagen, reticular, elastic) in a thin, almost fluid-like, ground substance. The latter is not preserved in routine preparations and accounts for some, but not all, of the spacing observed between the fibrous and cellular components. Areolar connective tissue forms the **stroma** that binds organs and the components of organs together. It forms helices about the long axes of expandable tubular structures, such as the gastrointestinal tract and other visceral organs, the ducts of glands, and blood vessels.

Fibers of connective tissue

Learning objectives for connective tissue fibers:

1. Be able to identify and distinguish between the three types of connective tissue fibers.
2. Be able to classify general connective tissues according to the arrangement of their extracellular fibers.

Collagen fibers are present in all connective tissues, vary in thickness from 1 to 10 μm and are of undefined length. In H&E preparations they stain a pink or pink-orange color. Because of the proteinaceous subcomponents these fibers, dependent on the dye used in staining, they can also be stained blue, green, yellow or red. Examine the dermis of skin (that region underlying the keratinized stratified squamous epithelium [epidermis]) for collagen fibers. Note the variation in size and the wavy, homogeneous appearance of the pink-orange staining collagen fibers. The majority of oval, densely staining nuclei in the field are those of associated **fibroblasts** that secrete and maintain the collagen fibers. The extent of the fibroblast cytoplasm usually cannot be seen in H&E preparations and what is actually visualized are fibroblast nuclei. Examine the external surface of a medium-sized (named) vessel for collagen fibers and fibroblasts. If available, examine a spread preparation of loose areolar connective tissue for collagen fibers and fibroblasts. The advantage of this type of preparation (usually a portion of a mesentery) is that it is not a section of tissue but rather an intact tissue, which is thin enough to allow the transmission of light. Examine the fibroblasts carefully, first noting their oval-shaped nuclei and then their associated cytoplasm. In these preparations the extent of the fibroblast cytoplasm can often be traced for considerable distances. Note that the fibroblasts lie immediately adjacent to, or on, collagen fibers, which stain lightly (a light pink in most preparations). Close observation of some fibroblast nuclei will reveal a light appearing strip crossing the blue-stained fibroblast nuclei. These strips are collagen fibers as seen against the stained chromatin background of the fibroblast nuclei. Return to a section of skin and re-examine the dermis with low power. Note, once again, that it forms a thick interwoven layer beneath the overlying epithelium. At increased levels of magnification note that the abundant thick, collagenous fibers are interwoven to form a compact network. The dermis is a classic example of **dense irregular connective tissue**. Next, a longitudinal section of tendon or ligament should be examined in detail. Note the regular, precise arrangement of collagen fibers into bundles that run parallel to one another. Fibroblasts are the primary cell type present and occur in rows parallel to the bundles of collagen fibers. Fibroblast nuclei usually are the only feature of these cells visualized and appear elongate and densely basophilic. Tendons and ligaments are classic examples of **dense regular connective tissue**.

Sketch and label the subcomponents of a region of dermis and tendon (or ligament). Illustrate the arrangement of collagen fibers and their association with fibroblasts. Examine an electron micrograph from a textbook illustrating the fact that each collagen fiber (type I) consists of banded unit fibrils, the smallest morphologically defined unit of collagen.

Elastic fibers appear as thin, homogeneous strands that are smaller and of more uniform size than collagen fibers. Usually elastic fibers cannot be distinguished easily in routine H&E preparations and require special stains (orcein or Verhoeff's elastic stain) to make them visible.

If a spread preparation of loose areolar connective tissue stained to demonstrate elastic fibers is available, examine it carefully and note darkly stained, variously sized, thin cylindrical fibers coursing across the field. These are elastic fibers. Look for an elastic fiber that has been broken. Because of their elastic properties, broken fibers will form a highly undulated snarl much like broken elastic fibers of clothing (eg. stockings). If a specifically stained preparation is not available, elastic fibers can be visualized to some degree using the same morphological criteria as demonstrated by special staining. However, in this case the elastic fibers stain the same color as collagen fibers but are narrower (thread-like), smooth and homogeneous in appearance, and of more uniform diameter than collagen fibers.

Examine a section of a named (muscular) artery for elastic tissue. Locate the lumen of the vessel and examine the region immediately beneath the lining endothelium. Note the highly scalloped, homogeneous layer of elastic tissue (the internal elastic lamina) at this location. If the vessel is specifically stained for elastin, move through the vessel wall and examine it for other dark-staining elastic fibers of various sizes. Return to the vessel interior. The internal elastic lamina is not a fiber *per se* but a thick homogeneous sheet of elastin. Now, and in the future, when additional arteries are encountered in routine H&E preparations, examine these vessels for the **internal elastic lamina**. In routine preparations this highly scalloped appearing membrane, although stained similar to collagen, has a slightly different refractive index. The appearance of the elastin can be made more visible by dropping the condenser of the microscope. Use the known position to locate and examine the negative image of the internal elastic lamina using this technique. Examine sections of any other tissue specially stained to demonstrate elastic fibers. Examine them in both longitudinal and transverse profiles. Note again the smooth, homogeneous nature of these darkly stained fibers. They often give a "copper wire-like" appearance when seen in sections of tissue.

Reticular fibers, like elastic fibers, are not seen in routinely prepared sections but can be demonstrated with silver stains or by the periodic acid-Schiff's (PAS) procedure. These are small fibers that form delicate networks and are a major component of the **stroma** that binds the cells of tissues and organs together. The most commonly used organs to demonstrate reticular fibers are the liver, kidney, spleen and lymph node where these fibers are especially prominent. With silver-stained preparations the reticular fibers stain black. Note the fine delicate network of fibers supporting the cellular components (**parenchyma**) of these organs.

Make a sketch of reticular fibers and their association with parenchymal elements. Compare these fibers with a sketch of elastic fibers.

Table 2. Key histologic features of connective tissue fibers.

Fiber type	Light microscopic appearance	Primary locations
Collagen fibers (type I collagen)	Coarse fibers 0.5-10.0μm in diameter, indefinite length, stain with protein dyes	Tendon, ligament, dermis, fascia, capsules, sclera, bone, dentin
Reticular fibers (type III collagen)	Delicate network of fine fibers, must be stained specifically to be demonstrated, usually by a reduction of silver or the periodic acid Schiff's (PAS) staining reaction	Stroma of lymphatic organs, bone marrow, glands, and adipose tissue
Elastic fibers	Smooth, homogeneous fibers of varying diameter, must be stained specifically to demonstrate well (orcein or Verhoeff's stain)	Dermis, lung, arteries, organs that expand

Cells of connective tissue

General connective tissue may contain a wide variety of cell types. Some are indigenous (residents) of connective tissues; others are transients and migrate to and from the general connective tissue from the vasculature.

Learning objectives for connective tissue cells:

1. Be able to distinguish and identify the following cell types (both indigenous and transient cells) found within connective tissues: fibroblasts, macrophages, plasma cells, fat cells, mast cells, neutrophils, eosinophils and lymphocytes.

Indigenous cells

Fibroblasts are the most common of the connective tissue cell types. They are large, spindle-shaped cells with elliptical nuclei. The boundaries of the cell are not seen in most routine preparations and the morphology and staining intensity of the nuclei vary with the state of activity. Active fibroblasts exhibit plump, light-staining nuclei; nuclei of inactive fibroblasts appear narrow and densely stained. Re-examine preparations of dermis, tendon (ligament) and areolar connective tissue and compare fibroblast nuclei.

Macrophages are abundant in general areolar connective tissue. They are commonly described as irregularly shaped cells with blunt cytoplasmic processes and ovoid or indented nuclei that are smaller and stain more deeply than those of fibroblasts. In actual fact, unless macrophages show evidence of phagocytosis, they are difficult to distinguish from fibroblasts. If special preparations are available, utilizing tissues from animals injected with India ink or trypan blue, examine the areolar connective tissue or liver preparations for cells that have phagocytized the materials injected. These will be macrophages. One location in which to examine macrophages in a "natural" setting is the center (medulla) of lymph nodes. Examine a routinely prepared lymph node under low power. Note that its central region is lighter staining and consists of anastomosing cords of cells separated by wide spaces. Examine the cords of cells and the adjacent spaces carefully for large rounded cells with brown-gold colored particulate material within their cytoplasm. These are macrophages.

Continue to look carefully within the interior of the medullary cords of the lymph node and note numerous **plasma cells**. These cells appear somewhat "pear-shaped" with small eccentrically placed nuclei in which the heterochromatin is arranged into coarse blocks forming a **clock face pattern**. The cytoplasm is basophilic and a weakly stained or light area of cytoplasm often appears adjacent to the nucleus on the side facing the greatest amount of cytoplasm. This light-staining area is referred to as a **negative Golgi image**. If available, examine a section of lactating breast. The connective tissue surrounding the secretory units of this gland often contains numerous plasma cells. Plasma cells also are present in large numbers in the connective tissue (lamina propria) of the intestinal tract. Examine the connective tissue that lies between adjacent intestinal glands for both plasma cells and small lymphocytes. The latter show a round, dense nucleus and only a scant rim of cytoplasm.

Fat cells are specialized for synthesis and storage of lipid. Individual fat cells may be encountered throughout the loose areolar connective tissue or may accumulate in large numbers to form fat (adipose) tissue. In routine sections, fat cells appear large, round and empty due to the loss of a stored central lipid droplet during tissue preparation. The remaining cytoplasm appears only as a thin rim around a large empty central space and if the nucleus is encountered in these large cells, it lies flattened on one side of the cell. Groups of fat cells have the appearance of chicken wire or a honeycomb. Fat cells are abundant in the hypodermis of skin - that region of tissue lying beneath the dermis. Fat cells are also common in the loose areolar connective tissue around the perimeter of a lymph node. If the lymph node preparation has been stained to demonstrate reticular fibers, examine the delicate network of reticular fibers enveloping each fat cell.

Mast cells are large, ovoid cells 20 - 30 μm in diameter with large granules that fill the cytoplasm. The nucleus is oval or round in shape and centrally located. Mast cells are present in variable numbers in loose connective tissue and often accumulate along small blood vessels. Examine a mesentery or areolar spread preparation for these large granulated cells. The granules can be stained different colors depending on the dye used. In tissues embedded in a plastic resin and stained with H&E, mast cell granules stain a light red color. If sections of this type are available, examine sections of the stomach and intestinal tract. In either organ the outer supporting muscular wall and adjacent connective tissue components should be examined for mast cells. They will appear as oval-shaped cells or cytoplasmic fragments of cells packed with coarse red-orange granules.

Transient cells derived from blood

Variable numbers of **leukocytes** constantly migrate into the connective tissues from the blood to carry out their specialized functions. **Neutrophils** are one type of leukocyte characterized by a multilobed nucleus and a faintly pink-staining cytoplasm. They are generally round in shape. Most often, the multilobed nucleus is the only prominent feature recognized when these cells are encountered in a section of general connective tissue. **Eosinophil** leukocytes also exhibit multilobed nuclei and are characterized by bright red (eosinophilic)-staining granules within the cytoplasm. **Lymphocytes** are smaller leukocytes (5 - 7 μm in diameter) characterized by a central, round nucleus surrounded by a thin rim of cytoplasm. In tissue, groups of lymphocytes appear only as collections of round, dark staining nuclei. Examine the outer, more darkly stained region (cortex), of a lymph node and thymus for these small darkly stained leukocytes. Examine the lighter-staining central region (medulla) of the thymus for mast cells and eosinophils.

Sketch and compare the various cell types encountered in general connective tissue.

Questions:

In sections of plastic embedded material stained with H&E, the cytoplasmic granules of both mast cells and eosinophil leukocytes stain red. How can these two cell types be distinguished from one another? Clue: consider their size and the nuclear profiles.

As plasma cells and lymphocytes are closely related, how do they differ morphologically?

Table 3. Key cytologic features of cells found in general areolar connective tissue.

Cell types	Nuclear characteristics	Cytoplasmic characteristics
Indigenous cells		
Fibroblasts	Oval, centrally placed, staining intensity variable depending on activity	Elongate, spindle- or stellate-shaped cell; usually not clearly distinguished is sectioned material
Unilocular fat cells (white fat)	Usually compressed at edge of cell, staining variable	Forms a thin rim around a single, large central lipid droplet
Multilocular fat cells	Central, spheroid, light staining	Numerous lipid droplets, abundant mitochondria
Mast cells	Central, spheroid to ovoid, may show abundant heterochromatin	Filled with secretory granules
Macrophages	Large, ovoid, most frequently indented	Light staining, contains phagocytosed material
Plasma cells	Usually eccentric, spheroid; heterochromatin clumps may form "clock face"	Basophilic, slate gray in color; may show negative Golgi image
Transient cells		
Neutrophils	Polymorphonuclear, 3-5 lobes common, chromatin dense	Light lilac staining granules
Eosinophils	Polymorphonuclear, 2-4 lobes common, chromatin dense	Bright red-orange granules fill the cytoplasm
Lymphocytes	Single, spheroid, abundant heterochromatin	Thin rim, light transparent blue staining

Cartilage

The classification of cartilage into hyaline, elastic or fibrous is based on the differences in the abundance and type of fiber within the matrix. **Fibers** and the **ground substance** constitute the **matrix** of cartilage.

Learning objectives for cartilage:

1. Be able to identify the three types of cartilage (hyaline, elastic and fibrous (fibro) cartilage) and their subcomponents.

Hyaline cartilage is the most common type of cartilage and forms the cartilages of the nose, larynx, trachea, bronchi, costal cartilages and the articular cartilages of joints. Examine the trachea or another tissue that contains hyaline cartilage. In the trachea, hyaline cartilage appears as a large homogeneous mass of tissue with a glassy appearance. The matrix appears homogeneous because the ground substance and the collagen fibers (type II) embedded within it have the same refractive index. Scattered within the light-staining, homogeneous cartilage matrix are small spaces called **lacunae**. These spaces contain the cells of cartilage known as **chondrocytes**. Chondrocytes generally conform to the shape of the lacunae in which they are housed. Note that deep within the interior of cartilage the cells and their lacunae usually exhibit a rounded profile whereas near the surface (edge) they are elliptical and flattened with the long axis oriented parallel to the surface. Near the center, chondrocytes often occur in small clusters called **isogenous groups**. The more intensely stained matrix immediately around chondrocytes is termed the **territorial matrix**. The less densely stained intervening matrix is called the **interterritorial matrix**. Except for the free surfaces of articular cartilages, hyaline cartilage is enclosed in a specialized connective tissue membrane (sheath) called the **perichondrium**. The outer region of the perichondrium is formed by a well-vascularized, dense irregular connective tissue. The region adjacent to the cartilage matrix is more cellular and the transition into cartilage is imperceptible. The perichondrial cells adjacent to the cartilage retain the capacity to form new cartilage.

Elastic cartilage is more flexible than hyaline cartilage and is found in the epiglottis, external ear, auditory tube and some of the small laryngeal cartilages. Elastic cartilage differs from hyaline cartilage chiefly in that the matrix contains an abundance of elastic fibers. Elastic fibers form a dense, closely packed mesh that obscures the ground substance deep within the cartilage, but, beneath the perichondrium, the fibers form a loose network and are continuous with those of the perichondrium. The elastic fibers of elastic cartilage, like those elsewhere, need to be specifically stained to be well demonstrated. Identify the perichondrium, matrix, lacunae and chondrocytes in elastic cartilage and compare them with similar structures found in hyaline cartilage.

Fibrous (fibro-) cartilage occurs in the symphysis pubis, intervertebral discs, in some articular cartilages and at sites of attachment of major tendons to bone. Fibrous cartilage **lacks a perichondrium** and merges into bone, hyaline cartilage or dense fibrous connective tissue. Fibrous cartilage represents a transition between cartilage and dense connective tissue. Typical chondrocytes enclosed in lacunae are found but only a small amount of ground substance is present in the immediate vicinity of the cartilage cells. **Chondrocytes** may **occur singly, in pairs or in short rows between** well defined bundles of **dense collagen fibers (type I)**.

Make a sketch of the three types of cartilage and label their subcomponents.

Bone

Two forms of bone can be recognized by visual inspection: compact (dense) bone and cancellous (spongy) bone.

Compact (dense) bone forms the solid, continuous mass that forms the perimeter of the named bones of the skeleton. **Cancellous (spongy bone)** is formed by an interlacing network of bony rods called **trabeculae**. These branch and unite to form a three-dimensional system of bony rods separated by small communicating spaces that form the marrow cavity. Bone is covered, except over articular surfaces and where tendons and ligaments attach, by a fibroelastic connective tissue membrane called the **periosteum**. A similar, less fibrous membrane, the **endosteum**, lines the marrow cavity.

Learning objectives for bone:

1. Be able to identify bone tissue and its subcomponents.

Compact bone

The initial examination of compact bone should be done using a ground preparation rather than a histological section of bone as the microscopic detail of the **matrix** is much more pronounced.

Bone is characterized by the arrangement of its matrix into layers called **lamellae**. Small, ovoid spaces, the **lacunae**, occur rather uniformly between and within lamellae, each housing a single bone cell called an **osteocyte**. Minute tubules called **canaliculi** radiate from each lacuna, penetrate and cross lamellae to join with canaliculi from adjacent lacunae. In compact bone, lamellae show three configurations. In transverse sections, most are arranged concentrically into several cylindrical units, much like growth rings of a tree. The concentrically arranged lamellae surround a central space known as a **Haversian canal**. Each unit, consisting of 8 to 15 concentric lamellae that surround the central Haversian canal, is referred to as an **Haversian system** or **osteon**. Portions of additional lamellae can also be visualized filling in the regions between osteon units. These are called **interstitial lamellae**. At the external surface, several lamellae course around the entire external circumference of the bone. These are called the **outer circumferential lamellae**. A similar but less well developed system of lamellae (one or two lamellae in thickness) lines the interior surface adjacent to the marrow cavity and these are referred to as the **inner circumferential lamellae**. Close inspection of individual osteons will reveal that they are outlined by a refractile line of modified matrix, the **cement line**. Note that the cement lines are not traversed by canaliculi. It must be emphasized that when viewing a preparation of ground bone (pieces of bone ground thin enough to permit the transmission of light) **only the matrix is observed** and the lacunar spaces and canaliculi within it. To visualize the cells of bone and the vascularized connective tissues associated with bone (periosteum and endosteum) a decalcified, histologically cut and stained section of bone must be used. Identify all the features observed previously using the preparation of ground bone in a histologic section of bone. Note that the matrix, at initial inspection, appears smooth, homogeneous and stains a light pink-red. It appears similar to hyaline cartilage. On closer observation, however, note the organization of the matrix. **Lacunae** contain **osteocytes** and are organized in a circular pattern around a **central Haversian canal**. Using low power, scan the section and identify several osteon units. By lowering the intensity of light and lowering the condenser note that the lamellae are crossed by fine, unstained canaliculi which can be traced to lacunae and give the latter a very irregular shape. Carefully examine these features under high power. Use of the fine focus adjustment, focusing back and forth through the section, may be required to see these structures. Carefully examine several Haversian canals. Note that they contain a delicate connective tissue that contains at least two small blood vessels. In well-preserved specimens a layer of flattened cells lines the limiting wall of the Haversian canal. These cells have osteogenic potential, ie, they can transform into bone-forming cells, osteoblasts, and can produce bone during the remodeling process. In addition, note the differences in diameter of the Haversian canals. Why is this? Is it related to the remodeling process? The delicate vascular connective tissue found within the Haversian canals is an extension of the endosteum, which also lines the marrow cavity. Compare the **endosteum** with the much thicker **periosteum** covering the external surface of bone. The outermost layer of the periosteum is dense irregular connective tissue with abundant collagen fibers, some elastic fibers, scattered fibroblasts and a network of blood vessels. The region of periosteum closest to bone is more cellular and consists of a loosely arranged connective tissue. Fibroblast-like cells immediately adjacent to the bone matrix are often called bone-lining cells or **osteoprogenitor cells**. If stimulated, they assume a cuboidal shape and synthesize and lay down new bone matrix. If this occurs these cells are termed **osteoblasts**. Some blood vessels leave the periosteum and enter the bone through **Volkmann's canals**. These are transverse canals that penetrate the bone from the endosteal and periosteal surfaces. Haversian canals on the other hand follow a longitudinal course parallel to the long axis of the bone and lie within osteons. Note that Volkmann's canals are not surrounded by concentric lamellae. Blood vessels passing through Volkmann's canals unite with those within the Haversian canals and link vessels in the marrow cavity and periosteum to the Haversian system. Large bundles of collagen fibers (**Sharpey's fibers**) enter the outer circumferential lamellae from the periosteum and firmly anchor the periosteum to bone. Re-examine both preparations of bone for both Volkmann's canals and Sharpey's fibers.

Sketch and label both a ground bone preparation and a section of decalcified bone. Compare and contrast the advantages and disadvantages of each preparation.

In addition to osteocytes, two other cell types are directly related to bone: osteoblasts and osteoclasts. Both are most easily found in young or developing bone and should be examined in detail.

Developing bone can form directly in a primitive connective tissue (mesenchyme) by a process known as intramembranous ossification or by replacement of a pre-formed hyaline cartilage model, the process of which is called endochondral ossification.

Learning objectives for developing bone:

1. Be able to describe the process associated with membrane bone formation and be able to identify the microscopic detail associated with a developing membrane bone.
2. Be able to describe the process associated with endochondral bone formation and be able to identify the microscopic detail associated with a developing endochondral bone.

Intramembranous ossification

Flat bones of the cranium and part of the mandible develop by intramembranous ossification and, as a result, are often referred to as **membrane bones**. Examine a section of forming membrane bone at low power and note that much of the bone is of the cancellous, or spongy, type. Carefully examine several trabeculae of the cancellous bone at higher power. Note the layer of low cuboidal, basophilic cells covering the external surface of each trabecula. These are **osteoblasts**. Examination of the interior of a trabecula will reveal **lacunae with osteocytes**. In the same region note a thin layer of bone matrix immediately adjacent to the cuboidally shaped osteoblasts that stains lighter than the remainder of the trabecular bone matrix. This layer of unmineralized matrix is called **osteoid**. After a short period it becomes mineralized to form true bone. As new osteoblasts are recruited from osteoprogenitor cells, which resemble fibroblasts, in adjacent mesenchymal tissue, they produce osteoid and eventually become enveloped in their own matrix. When this occurs the osteoblasts are termed osteocytes. With development, the trabeculae continue to thicken by the addition of new bone on their external surface (appositional growth) and the spaces between some trabeculae are gradually obliterated. As bone growth encroaches on vascular spaces within the surrounding mesenchymal connective tissue, matrix is laid down in irregular, concentric layers around blood vessels to form **primary osteons**. The entrapped blood vessels and connective tissue form the contents of the developing, primitive Haversian canals. These newly formed osteon units then undergo remodeling.

Identify and sketch several fields comparing one with the other and envision primary osteon formation.

Endochondral bone formation

Bones of the extremities, pelvis, face, base of skull and vertebral column result from endochondral bone formation, a process that involves simultaneous removal of a precursor hyaline cartilage model and formation of bone matrix. Cartilage does not contribute directly to the formation of bone but much of the process is concerned with the removal of the cartilage precursor. Initial indications of ossification of a long bone occur at the center of the cartilage model in the shaft or diaphysis. In this area, the primary ossification center, chondrocytes hypertrophy, their lacunae expand and the matrix between adjacent lacunae is reduced to thin, fenestrated partitions. Simultaneously, the perichondrium becomes more vascular and assumes an osteogenic function. A thin layer of bone called the **periosteal collar** forms around the perimeter of the altered cartilage and acts as a temporary splint. Blood vessels from the former vascularized perichondrium (now best termed periosteum) invade the degenerating cartilage as a **periosteal bud**. The connective tissue sheath that accompanies the invading blood vessels contains cells with osteogenic properties. As the cartilage matrix breaks down, lacunar spaces are opened up, become confluent, and narrow tunnels in the calcified cartilage matrix are formed. Blood vessels grow into these tunnels bringing with them osteogenic cells that align themselves on the surfaces of the calcified cartilage. The latter differentiate into osteoblasts and begin to lay down new bone matrix (osteoid). Thus, early trabeculae consist of a core of calcified cartilage covered by a shell of bone. These are removed through the activity of osteoclasts that appear on the bony shell. In this way, an expanding cavity is formed in the developing shaft. Support is provided by expansion of the periosteal collar, which becomes thicker and longer as the periosteum lays down new bone at the external surface. The process continues as an orderly progression toward **both ends** of the cartilaginous precursor. Several zones of activity can be distinguished in the remaining cartilage. Beginning at the ends furthest from the primary ossification center, these are as follows:

Zone of reserve cartilage. This area consists of typical hyaline cartilage with chondrocytes and their lacunae randomly arranged throughout the matrix.

Zone of proliferation. In this region chondrocytes actively proliferate and as a result chondrocytes become aligned in rows or columns separated only by a small amount of matrix.

Zone of maturation and hypertrophy. Here, cell division stops and chondrocytes mature and enlarge. Lacunae expand at the expense of the intervening matrix and the matrix between adjacent rows of chondrocytes becomes even thinner.

Zone of calcification and cell death. The matrix between and around the rows of chondrocytes becomes calcified and chondrocytes die, degenerate and leave empty lacunae.

The thin regions of matrix between lacunae then break down resulting in irregularly shaped tunnels appearing in the matrix. The extent of this zone can be recognized by careful examination of the matrix, which stains slightly darker than that of the previous zones indicating the extent of calcium diffusion into the matrix.

Zone of ossification. Vascular connective tissue enters the tunnel-like spaces formed in the adjacent zone and provides osteoprogenitor cells that differentiate into osteoblasts. **Osteoblasts** gather on the surface of the calcified cartilage and lay down bone matrix (osteoid). Examine these cells carefully.

Zone of resorption. The calcified cartilage and the bony covering are resorbed due to the action of osteoclasts. In this way the marrow cavity increases in size as the developing bone increases in length. **Osteoclasts** are large multinucleate giant cells with a moderately stained acidophilic cytoplasm. Some may contain as many as 30 nuclei. Individual nuclei show no unusual features and usually are located in the part of the cell furthest from the bone surface. The cell surface adjacent to the bone may show a striated (ruffled) border. Osteoclasts reside in shallow depressions on the surface of bone called **Howship's lacunae**.

Carefully examine and sketch the zones within the cartilage for the details described and then re-examine the periosteal collar.

Simultaneous with these events the periosteal collar increases in thickness and length, extending towards the ends of the developing bone. Its growth continues to provide a splint around the area of weakened cartilage.

Near the time of birth, new centers of ossification (epiphyseal or secondary ossification centers) appear in the epiphyses. Hyaline cartilage of the epiphyses show the same sequence of events as observed in the diaphysis, but growth and subsequent ossification spreads simultaneously in all directions. Ultimately, all the cartilage will be is replaced by bone, except for the free end in the joint cavity, which remains as articular cartilage. Hyaline cartilage also persists as a narrow plate between the diaphysis and epiphysis called the **epiphyseal plate**. Its continued growth permits further elongation of bone. Note that the epiphyseal plate continues to exhibit the various zones associated with endochondral bone formation. When growth in the cartilage plate ceases, it is replaced by bone and further increase in length of the bone is no longer possible. Thus, the rapid growth of a long bone is largely the result of cartilage growth and bone replacement and not growth of bone *per se*. Examine the external surface of the periosteal collar for a layer of osteoblasts and its associated osteoid.

Sketch this region, including its relationship with the surrounding periosteum.

Table 4. Key histologic features used in identifying different types of compact connective tissues.

Type	Arrangement of cells and matrix	Additional features
Hyaline cartilage	Glasslike matrix; lacunae with chondrocytes randomly arranged, slitlike in appearance near perichondrium	Isogenous groups, territorial matrix
Elastic cartilage	Matrix more fibrous in appearance; elastic fibers (need to be stained selectively); lacunae with chondrocytes randomly arranged	Large isogenous groups
Decalcified bone	Lacunae with osteocytes show organization within lamellae of osteons; Haversian canals lined by endosteum contain blood vessels	Tide marks, bone marrow, irregular shape of lacunae
Ground bone	Matrix only arranged into distinct Haversian systems; interstitial and circumferential lamellae	Canaliculi distinct between lacunae
Fibrous cartilage	Dense fibrous appearance with a small amount of ground substance; round cells within lacunae are arranged in short rows between collagenous fibers	Round-shaped chondrocytes
Tendon, ligaments, aponeuroses	Fibroblast nuclei dense staining and elongate, lie in parallel rows between regularly arranged collagen fibers	Lacunae absent
Dermis, capsules of organs, periosteum, perichondrium	Fibroblast nuclei dense and elongate; randomly scattered between dense interwoven, irregular arrangement of collagen fibers	Lacunae absent

Three types of muscle can be distinguished: skeletal, cardiac and smooth.

Learning objectives for muscle tissue:

1. Be able to distinguish between the three types of muscle.

Skeletal muscle

The skeletal muscle cell (fiber) is a giant cell that ranges between 10 and 100 μm in diameter and is extremely variable in length. Skeletal muscle cells are **multinucleated** and may contain several hundred nuclei. All are peripheral in location, evenly spaced immediately beneath the plasmalemma. The nuclei are elongated in the direction of the long axis of the cell. Chromatin tends to be distributed along the nuclear envelope and one or two nucleoli are usually present. The most outstanding structural feature of the skeletal muscle cell is the presence of alternating light and dark **bands** or **cross-striations** that are visible when the cell is viewed in longitudinal section. The **dark bands** are called **A bands** and the **light bands** are called **I bands**. Running transversely through the center of the I band is a narrow dense line, the **Z line**. Myofibrils are elongated, thread-like structures that fill the skeletal muscle cell, compressing the nuclei to a peripheral location. **Myofibrils** are the smallest units of contractile material that can be identified with the light microscope and in transverse section appear as small, solid dots within the muscle cell (fiber). Each myofibril shows the identical banding pattern to that of the whole cell. Indeed, the banding of the skeletal muscle cell results from the bands on the contained myofibrils being in perfect alignment as the plasmalemma of the cell is transparent. Compare the banding seen with the light microscope with that visible in an electron micrograph.

The interior of the tongue and lip are excellent locations to carefully examine the details of individual skeletal muscle cells. Skeletal muscle is closely associated with connective tissue at all levels of organization. In examining a named muscle, the entire muscle is surrounded by a connective tissue sheath called the **epimysium**. Septa pass from the deep surface of the epimysium to envelop muscle fascicles (groups of muscle cells) as the **perimysium**. A delicate connective tissue wraps each individual muscle cell as an **endomysium**. Although given different names according to its association with different structural units of skeletal muscle, the connective tissue forms a continuum and acts not only to bind the various muscle units together but also functions as a harness and aids in integrating and transmitting the forces of contraction. It consists of collagenous, reticular and elastic fibers and contains several connective tissue cell types, the most common of which are fibroblasts. The endomysium is delicate and consists primarily of reticular and thin collagen fibers. It contains blood capillaries and small nerve branches. Larger nerves and blood vessels lie within the perimysium.

Sketch and label longitudinal and transverse profiles of skeletal muscle cells. In the transverse profile include its association with the surrounding connective tissue.

Cardiac muscle

Cardiac muscle is associated with the heart and forms the majority of the heart wall or myocardium. Cardiac muscle cells are small cylindrical cells that **branch** and are linked to one another, end to end, by specialized junctions known as **intercalated discs**. The latter appear as darkly stained transverse lines. Each cardiac muscle cell contains one or occasionally two **centrally positioned nuclei**. The same banding pattern witnessed in skeletal muscle occurs in cardiac muscle. Although A bands, I bands and Z lines are visible they are not as conspicuous as those of skeletal muscle. Myofibrils are fewer in number and often grouped into bundles that diverge around nuclei. As a result, regions of cytoplasm appear structureless at each nuclear pole. Examine both longitudinal and transverse profiles of cardiac muscle cells to confirm the position of their nuclei. A web of reticular and fine collagenous fibers is present between cardiac muscle cells and corresponds to an endomysium. Numerous capillaries are present in this layer of connective tissue.

Sketch and label both longitudinal and transverse profiles of cardiac muscle cells. The longitudinal profile should include an intercalated disc. Compare these light microscopic observations with those features seen in electron micrographs of cardiac muscle.

Smooth muscle

Smooth muscle is widely distributed and plays an essential role in the function of organs. It forms the contractile portion of the walls of blood vessels, hollow viscera, such as the digestive, respiratory, urinary and reproductive systems, and is a subcomponent of most other organs.

Smooth muscle cells are shaped like **elongated spindles** with a **single**, **central nucleus** occupying the wide portion of the cell midway along its length. The nucleus is elongated in the long axis of the cell. Smooth muscle cells lack the cross-striations observed in the two other forms of muscle.

Smooth muscle cells may be present as small isolated units or may from prominent sheets. In any one sheet of smooth muscle, the cells tend to be **oriented in the same direction** but are offset so that the wide portions of some cells lie adjacent to the tapering ends of neighboring cells. This is most evident in transverse sections, where the outlines of the cells vary in diameter according to where along their lengths the cells were cut. Nuclei are few in this view and present only in muscle cells cut near their largest profiles (centers). A thin connective tissue of fine collagenous, reticular and elastic fibers aids in binding the smooth muscle cells into bundles or sheets. In routine preparations this fine reticuloelastic sheet is almost imperceptible and the field is dominated by profiles of smooth muscle cells.

Examine and sketch the muscle wall of a transversely cut segment of small intestine and note that it consists of two sheets or layers of smooth muscle. The smooth muscle cells in the external layer will be sectioned transversely, those of the adjacent inner layer will be cut parallel to their long axis. Examine the smooth muscle cells carefully at higher magnifications and note the position of their nuclei.

Next, examine the wall of any named artery for its smooth muscle layer, which occupies the center of the vessel wall. What is the orientation of cells within this smooth muscle layer? How can smooth muscle cells be differentiated from adjacent fibroblasts?

Table 5. Key histologic features that distinguish muscle types.

Type	Cell shape	Nuclei	Striations	Other features
Skeletal	Long cylinders	Peripheral, multiple	Present	Endomysium, perimysium
Cardiac	Short branching, anastomosing cylinders	Central, usually single, occasional double	Present	Intercalated discs
Smooth	Small spindles	Central, single	Absent	Cells packed tightly together, occurs in sheets, layers or bundles with nuclei oriented in the same direction