COMPARATIVE HISTOLOGY
VANB602

HISTOLOGY LABORATORY MANUAL

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LAB 1 EPITHELIUM

 # 2 - umbilical cord
 # 95 - thyroid gland
 # 65 - gallbladder
 # 52 - jejunum
 # 69 - esophagus/trachea
 # 80 - boar epididymis
 # 41 - esophagus
 # 36 - lip
 # 33 - planum nasolabiale
 # 75 - ureter
 # 76 - bladder
 # 83 - urethra
 # 91 - mammary gland
 # 93 - teat sinus or canal

1. **Simple Squamous Epithelium**. **Slide 2** is a section of the **umbilical cord**. Hold the slide to the light and observe that there are up to 5 **lumens** present. One is the **urachus**, a functionless remnant of part of the duct of the allantois of the embryo. It extends from the bladder to the umbilicus. The other lumens are the **umbilical arteries and veins**. Examine these at this time. In general, the lumens of blood vessels and lymphatic vessels are lined by a single layer of flat epithelial cells known as **endothelium**. Endothelium is the type of simple squamous epithelium that lines vessels. Look at the endothelium of the large umbilical vessels as well as the many smaller vessels in the area. Only the nuclei, which bulge into the lumen, are visible.

2. **Simple Cuboidal Epithelium**. **Slide 95** is a section of the **thyroid gland**. **Thyroid follicles** surround lumens that may be filled with a homogeneous **colloid** (dark pink in color). Note that each follicle is lined by a *single* layer of cells that are about as wide as they are tall. This epithelium is considered **simple cuboidal**.

3. **Simple Columnar Epithelium. Slide 65** is a section of the wall of the **gallbladder**. **Slide 52** is a section of the **jejunum**, a part of the small intestine. The epithelium lining the gallbladder and the jejunum consists of a single layer of cells whose height exceeds their width; therefore, this epithelium is considered **simple columnar**.

Again, note that each epithelium lining surrounds a lumen. Some versions of slide 52 have large intestinal parasites that occupy the center of lumen. These are, of course, *not* a normal part of jejunal tissue and should be ignored for the time being.

The epithelium in the intestine covers the **villi,** which are seen as finger-like projections into the lumen. Notice that the nuclei of the jejunal epithelium form a single row and that the cells are tall and thin. The lumenal, or apical side of the epithelium has a **striated border**, formed by **microvilli** on the apical domains of the epithelial cells. This is true for both the gallbladder and the small intestine. Other cell types may also be found in the epithelium of the jejunum, such as goblet cells. These will be discussed further in later labs.

4. **Pseudostratified Columnar Epithelium**. Pseudostratified columnar epithelium consists of basal cells and columnar cells. Both cell types rest on a basal lamina, but only the columnar cells reach the lumen. Since the nuclei of these two cell types are at different levels, this gives the misleading impression that the cells are layered, hence the name pseudostratified.

Slide 69 is a section of the **trachea** and **esophagus**. Find the lumen of the trachea and look for the epithelial cells which immediately surround it. Respiratory epithelium in the larger airways is **ciliated pseudostratified columnar**. Look at the epithelium of the trachea and observe that most, but not all, of the cells are tall and narrow and that their nuclei seem to be on different levels. However, all of the cells contact the basal lamina (though this may not be viewable). Examine **slide 80**, the **boar epididymis** and study the epithelial lining of the duct of the epididymis, which has been sectioned in many different ways. This is an example of **pseudostratified columnar epithelium with stereocilia** (you should be aware that the term "stereocilia" is really a misnomer: stereocilia are actually a type of microvilli). Recall that the epididymis is part of the male reproductive system and stores sperm. The long thin cells which occupy the lumens in slide 80 are spermatozoa.

5. **Stratified Squamous Epithelium**. **Slides 4l & 69** contain sections of the **esophagus** of the dog and rabbit, respectively. **Slide 36** is a section of the **lip** which has **skin** on one edge and an **oralmucous membrane** (i.e., the inside of the lip) on the other. **Slide 33** is a section of the **planum nasolabiale**, the broad hairless area between the nostrils of a cow's muzzle. The epithelium of all these surfaces is comprised of several layers of cells that become flatter near the surface. The superficial cells of the epithelium of wet surfaces (mucous membranes) e.g., the esophagus and oral cavity, usually retain their nuclei. The superficial cells of the epidermis of the skin, e.g., the planum nasolabiale, have lost their nuclei. Cells in **stratified squamous epithelium** are attached to one another by numerous **intercellular bridges**. These spine-like cytoplasmic processes (known as tonofilaments, which are a type of intermediate filament) anchor back to the desmosomes (not viewable) within the cell, helping to link the cells together. The intercellular bridges are visible by light microscopy. Look for them in slide 33, and refer to Fig. 12.10, #5 on p. 91 of your Histology Atlas (Bacha, 2nd ed.).

Directly underlying the epithelium is another layer of tissue known as **connective tissue** (to be discussed further in later labs). Finger-like projections of pale, pink **papillae** (loose connective tissue) extend up to the epithelium of the skin. The plane of section sometimes does not include their entire length. Therefore, what appear to be isolated patches of pale pink tissue scattered through the epithelium are actually pieces of underlying papillae from the connective tissue.

6. **Transitional Epithelium**. This special epithelium is layered, nonabsorptive, stretchable, and found primarily in the urinary passages. The apical cells can be either flat (squamous) or balloon-shaped depending on the

degree of distention of the lumen. When the lumen is full (e.g., with urine), the epithelium stretches and distends, causing the cells to flatten. When the lumen is empty, the cells become more cuboidal or balloon-shaped.

Slides 75, 76, and 83, are sections of the urinary tract at different levels: **ureter**, **bladder**, and **urethra**, respectively. Look for the transitional epithelium around the central lumen of these slides and try to determine whether the lumen was full or empty (of urine) when these slides were made, based on the morphology of the epithelium. Reexamine the urachus on slide 2. Can you find an example of transitional epithelium?

7. **Stratified Cuboidal and Columnar Epithelium**. **Slides 91 and 93**. **Stratified columnar epithelium** generally occurs in intermediate zones between a simple epithelium and a stratified squamous epithelium. It is located in parts of the larynx and pharynx, and in ducts of large glands. It is not commonly seen on your slides, but examples may be present on occasion. Stratified cuboidal epithelium is found in the ducts of sweat gland and also in the intermediate zones of the anal canal, conjunctiva (eye), and female urethra. A two-layered (a.k.a. bistratified) cuboidal epithelium lines the interlobular ducts of the **mammary gland**. Examine **slide 91** for an example of bistratified cuboidal epithelium.

Slide 93 will either show the **teat sinus of the cow** (present in half of the student slide boxes) or the **teat canal of the cow** (present in the remaining half of the student slide boxes), depending on which one is in your particular slide box. Both regions are part of the mammary gland, but the teat canal is a more distal portion of the gland than the teat sinus, i.e., the teat canal is closer to the outer skin. Based on this, which slide – that of the teat sinus or the teat canal – would you predict will show the thicker epithelial lining (and why)?

Which one do you have? To determine this, examine the epithelium immediately surrounding the large lumen in the *center* of the tissue. The slide of the **teat sinus** has a bistratified (2 layers) epithelial lining whose cells vary in shape from **bistratified columnar** to **bistratified cuboidal**. Whereas the slide of the **teat canal** is lined by a **stratified squamous epithelium**. Consult the illustrations immediately following the text of this lab, or look at Fig. 12.58 and 12.59 on p. 104 of your Histology Atlas (Bacha, 2nd ed.) for a reference to the teat sinus, and Fig. 12.61 on p. 105 for a reference to the teat canal.

Please be sure to share your slide with your classmate and vice versa so that you see examples of both tissue types.

LAB 2 SKIN AND ASSOCIATED STRUCTURES

 # 27 - skin (horse)
 # 33 - planum nasolabiale
 # 34 - skin (dog)
 # 35 - skin, ear (dog)
 # 36 - lip (dog)
 # 93 - teat
 # C-30 - skin (avian)

Slides 27 (horse), 33 (cow, planum), 34 (dog), 35 (dog, ear) and 36 (dog, lip) are sections of skin. All but slide 33 are of thin skin with hair. Slide 34 provides a comparison between the dorsal cervical (neck) and ventral abdominal skin of the dog. As you look at the histological appearance of skin in these slides make note of the following:

1. **Epidermis**. Epidermis is keratinized stratified squamous epithelium. Examine **slides 33 and 93**. Identify the **4 layers of keratinocytes** discussed in lecture. Make sure you can recognize the **stratum basale (stratum germinativum), stratum spinosum, stratum granulosum, and stratum corneum**. The stratum granulosum is better represented in slide 93, the cow teat. Notice the cytologic features of each layer, including: cell shape, melanin granules (in the stratum basale), intercellular bridges (particularly in the stratum spinosum)**,** keratohyalin granules (particularly in the stratum granulosum) and the presence or absence of nuclei.

2. **Dermis**. The dermis is composed of two layers. The superficial layer is the **papillary layer**, the deeper layer is the **reticular layer**. The papillary layer is thin and composed of **loose connective tissue**. We will discuss connective tissue in more detail in the upcoming weeks. Note that the loose connective tissue consists mostly of scattered cells of various types (including the nuclei of fibroblast cells), and an abundance of extracellular matrix (ECM). ECM includes a loose network of fine fibers (mainly collagen) and ground substance.

Notice the **dermal papillae**, the finger-like projections of the dermis which interdigitate with the overlying epidermal ridges. These papillae are important in anchoring the epidermis to the dermis. Papillae are prominent in hairless skin, such as slide 33, but not in skin that has hair. In addition to fibroblast cell nuclei, you may see other connective tissue cell types (to which you will be introduced in upcoming labs) here including mast cells, macrophages, extravasated neutrophils (extravasated = forced from the bloodstream into the surrounding tissue), and lymphocytes. We will discuss how to identify these cells in later labs.

The **reticular layer** is the thicker, deeper layer of the dermis. It is composed of **dense irregular connective tissue**. Dense connective tissue is characterized by having more collagen fibers in the ECM (larger, coarser, and often bundled) when compared to the amount of collagen seen in loose connective tissue of the papillary layer. Dense connective tissue also tends to contain fewer cells. Sweat glands, hair follicles, and sebaceous glands tend to be found in this layer. Also, notice the many **blood vessels** in the dermis.

3. **Subcutis, Hypodermis or Subcutaneous Connective Tissue**. This layer is present *in theory* on all the slides, but it will depend on how deep your tissue layer is sectioned. The hypodermis is composed mainly of **loose connective tissue and adipose tissue**, which gives the skin flexibility and free movement over the underlying structures. Adipose (fat) cells are usually present in this layer, though none may be seen in slide 33. Large fat deposits in the subcutis are characteristic of the fat pads and digital cushions where they serve as shock absorbers. In slide 33, the subcutis (which may not be present in your slide) contains **serous glands** (these glands will be covered in the next lab) that keep the nose moist.

4. **Hair and Hair Follicles**. Because these structures are sectioned at different levels and angles there is a great variety in their appearance. Try to identify the parts that are visible. The **hair** itself is usually present and is pigmented. You can often see a **medulla** and **cortex**. While the **inner root sheath** is often not discernible, the **outer root sheath** is consistently present. The **dermal (connective tissue) sheath** is also not well portrayed except around sinus hairs where it is well developed. **Sinus hairs** (e.g. whiskers, or vibrissae) are highly specialized for tactile sense and can be seen on slide 36 – the dog's lip. If your slide does not show this structure adequately, check your neighbor's. In this structure a **blood-filled annular sinus** separates the inner and outer layers of the thickened dermal sheath. Special staining would reveal a rich nerve supply surrounding these hairs which are very sensitive to any slight pressure applied to them. Study the hair follicles on slides 27, 34, 35, and 36. **Note that the follicles of the dog are compound while those of the horse are simple**.

5. **Skin glands**. Identify **sebaceous** and **sweat glands** on slides 27, 34, and 36. Both types of glands are near hair follicles. It may be possible on some sections to observe the point at which some of these glands open into the hair follicles. Further details on glands of the skin will be covered in the exocrine gland lab.

6. **Arrector Pili Muscle**. You should be able to recognize this strip of **smooth muscle**, especially on slide 27 where it is usually well represented, as a thin pink bundle of cells, with elongated nuclei, passing through the dermis near a hair. The contraction of these smooth muscles promotes the erection of the hair shaft into a more vertical position.

7. From your avian slide box, examine **slide C-30**, a section of avian skin. **Avian skin lacks glands**. The uropygial or preen gland at the base of the tail provides oil in the absence of sebaceous glands in the skin. In addition, **feather**

follicles rather than hair follicles are present. Note that the epidermis, dermis, and subcutis of feathered, avian skin is very similar to that of haired, mammalian skin. The structure of the feather follicle corresponds to that of the hair follicle. There exists a dermal and an epidermal portion, as well as an inner and outer epithelial sheath and a connective tissue sheath. The feather muscles are comparable to the arrector pili muscles; they elevate feathers or pull them closer together.

LAB 3 GLANDS

 # 52 - jejunum
 # 34 - skin (dog)
 # 36 - lip
 # 27 - skin (horse)
 # 59 - parotid gland
 # 60 - submandibular gland
 # 61 - sublingual gland
 # 39 - infraorbital sinus

Glands are structures that release a product(s). The latter is most generally a substance, which has been synthesized by the secretory cell or cells, but it may, in some cases, consist of whole or disintegrated cells. Saliva, mucus, enzymes and hormones are examples of synthesized products, while spermatozoa, blood cells and sebum are examples of whole and disintegrated cells, respectively. Although most glands are multicellular, some are unicellular and are located amongst the nonsecretory cells of an epithelium. Multicellular glands that retain communication with an epithelial surface by an excretory duct are designated exocrine glands. Glands which have lost communication with an epithelial surface and which secrete directly into the surrounding tissue fluid are called endocrine (ductless) glands. Some glands have both exocrine and endocrine capabilities, e.g., pancreas and liver. Multicellular exocrine glands may be classified as simple or compound depending upon the degree of complexity of their duct systems. The secretory units (groups of secretory cells) of a simple multicellular gland discharge their product directly to the epithelial surface or to the surface of a single unbranched excretory duct. The duct system of a compound gland, alternatively, is elaborate and highly branched. The secretory units of either type of gland may be organized into tubes (tubular), saclike structures (alveolar or acinar), or have a flask shape (tubuloacinar).

Compound glands usually are divisible into **lobes**, which are divided into smaller units called **lobules**. The main excretory duct branches to each lobe. These branches are called interlobar ducts before entering a lobe. Once within the lobe these major branches are called **intralobar ducts**. Branches of the latter go to each lobule and are called **interlobular ducts**. Upon entering the connective tissue of the lobule, the latter become known as **intralobular ducts**. Intralobular ducts branch repeatedly, eventually terminating in a secretory unit.

Secretory products may be released from secretory units in different ways. The merocrine release is typical of most glands. Here secretory vesicles (small, membrane bound spherical sacs containing secretory product) attach directly to the plasma membrane and discharge their contents to the exterior. In the apocrine release, the secretory product, a small amount of surrounding cytoplasm and a bit of plasma membrane, is pinched off from the surface of the secretory cell. In the holocrine method of release, the entire secretory cell becomes the product. Sebaceous glands are classic examples of the holocrine method. Here the entire secretory cell, filled with product, disintegrates and is released from the gland.

The testes, bone marrow, and ovary all produce and release cells and, therefore, may also be designated holocrine glands.

Secretory glands from the digestive, respiratory, and reproductive systems are ordinarily comprised of **serous or mucous secretory cells**, or of a combination of the two. **Serous cells** produce a thin, watery secretion. Morphologically, they are wedge-shaped and possess a round nucleus. They may be the exclusive cell type found in a tubular, alveolar, or tubuloalveolar secretory unit. **Mucous cells** frequently are pear-shaped, but may be wedge-shaped sometimes. Their cytoplasm appears "frothy" and their nucleus often lies flat against the basal end of the cell. They, too, may be organized into tubular, acinar, or tubuloacinar secretory units.

Today's lab will cover the goblet cell, a unicellular gland, as well as several different multicellular exocrine glands. Additional exocrine glands and endocrine glands will be studied during subsequent laboratory sessions.

1. **Goblet Cells**. These cells are found, among other places, in various parts of the digestive and respiratory systems, where they occur within epithelia lining various parts of the system. Typically, about one third of the basal end of the cell is attenuated while the apical portion is expanded resembling the bowl of a goblet, hence the name. Numerous secretory vesicles fill the bowl of an active cell giving it a frothy appearance. The vesicles are filled with mucigen, a glycoprotein which forms mucin upon taking up water. The nucleus of the cell is generally located in the cytoplasm between the stem and the bowl of the "goblet".

Examine **slide 52 (jejunum)** and look for examples of **goblet cells** in the epithelial lining. The mucus produced upon release of the mucigen is a clear viscous substance consisting of mucin, various salts, epithelial cells and leukocytes. In the digestive tract, it serves as a lubricant; whereas in the respiratory tract it serves to trap dust particles.

2. **Sweat glands** are **simple, coiled tubular glands**. Examples can be found on **slides 34 and 36 (dog) and 27 (horse)**. The tubular secretory unit is coiled and composed of **cuboidal (sometimes flattened) cells** surrounding a rather large lumen. They are found in the dermis or upper hypodermis appearing, usually as clusters of round, oval, or elongated cavities. The secretory unit leads into an **excretory duct** which is also lined by **cuboidal cells** and which empties into the upper portion of a hair follicle or less frequently directly to the skin surface. In favorable sections, portions of excretory ducts may be seen. Why do you only see portions of a coiled tubular gland or its excretory duct as a rule?

In humans, the principal sweat gland is a coiled, tubular, merocrine gland (also referred to as eccrine sweat glands). They are found all over the body, and produce sweat as a means of body temperature regulation. Merocrine sweat glands are found also in the foot pad of the cat and dog and the carpus of pigs.

Apocrine sweat glands also occur, however, in the pubic, perianal, and axillary regions, and in addition to producing sweat in these regions, they also produce an odor, which is amplified by bacteria that use the sweat as a nutrient source. These apocrine sweat glands are also known as scent glands.

The principal sweat gland of domestic animals has traditionally been considered an apocrine gland (whereby a portion of the secreting cell's body is lost during secretion). However, more recent evidence from electron microscopy has cast doubt on this interpretation and they are now considered merocrine glands (whereby cells secrete their substances by exocytosis), though the apocrine name is still used. These glands are quite active in the horse but produce little secretion in other species.

3. **Salivary Glands**. The major salivary glands are **compound, tubuloacinar glands** which lie outside of the mouth cavity. Their excretory ducts open into the mouth cavity. These are merocrine glands with secretory units consisting exclusively of serous or mucous cells, or of a mixture of the two cell types. Myoepithelial cells surround the secretory units.

A section through a portion of the **parotid gland, slide 59 (horse)**, will show numerous **lobules** separated by **connective tissue septa**. **Blood vessels and interlobular ducts** can be seen in the connective tissue. The interlobular ducts have a **bistratified columnar or cuboidal epithelium**. They drain from intralobular ducts. Intralobular ducts can be found *within* the lobule. **Striated ducts,** (the larger of the intralobular ducts) are lined by a **simple columnar epithelium**. The fine striations, located between the nucleus and basement membrane, are formed from deep infoldings of the basal plasma membrane and from mitochondria. Striated ducts drain from **intercalated ducts** (the smaller of the intralobular ducts). They are lined by cuboidal cells and terminate at the secretory units.

So, to restate the order: the product from the acinus drains first into an intercalated duct, lined by a simple cuboidal epithelium. Intercalated ducts drain into striated ducts, which are lined by simple columnar cells. Striated ducts drain into interlobular ducts.

Note the shape and cytoplasmic characteristics of the **serous cells**, which make up the secretory units. They are wedge-shaped and are arranged around the tiny lumen of each tubuloacinar unit. The cytoplasm of the basal end of the cell is basophilic while that of the apical end is acidophilic and filled with secretory granules. The nucleus is round and is located in the basal half of the cell. What causes the basophilia in these cells? Although the secretory units of the parotid gland usually consist exclusively of serous cells, mucous cells occur in some animals, e.g., carnivores and lambs.

The **submaxillary (submandibular) gland (slide 60, horse)** is similar to the parotid in structure. However, it is a **mixed gland** whose secretory units are composed of both serous and mucous cells. The serous cells are similar to those seen in the parotid. The mucous cells can be recognized by i) their pale, frothy appearance caused by the presence of numerous secretory vesicles, and by ii) the position of the nucleus which generally lies flat against the basal portion of the cell. **Striated and intercalated ducts** are present. The submandibular is also a mixed gland in the dog, ruminants and man but is a serous gland in rodents.

The **<u>sublingual gland (slide 61, horse)</u>** is a **mixed gland** in small carnivores, horses, and people; but in pigs, cows, and sheep it is almost entirely a mucous gland.

Serous demilunes (crescent-shaped caps of serous cells) can be seen on the surface of mucous acini in both the submaxillary and sublingual glands. Serous cells may also be seen amongst the mucous cells of some acini. **Striated and intercalated ducts** are present.

Located between the basement membrane and the basal end of the secretory cells of salivary glands (also sweat and mammary glands) are **myoepithelial cells**. The arm-like processes of these contractile cells surround the secretory units. Contraction of these processes squeezes secretion from the secretory unit. Usually, only the flattened nucleus of myoepithelial cells can be seen in typical histologic preparations.

4. **<u>Sebaceous Glands</u>**. These **holocrine glands** (the entire cell disintegrates to secrete its substances) usually empty into the upper portion of a hair follicle; but in places such as the lip, clitoris, and glans of the penis, which are absent of hair, they open directly onto the epithelial surface. Within the gland, mitotically active stem cells lie along the outer edge of each **alveolus**. These cells give rise to cells that move into the interior of the alveolus where they synthesize a lipid secretory product. These secretion-laden cells eventually die, disintegrate, and are liberated into the excretory duct as an oily secretion known as **sebum**. In humans, sebaceous glands are found everywhere except the palms of the hand and soles of the feet.

Reexamine slides 27, 34, and 36 and locate the sebaceous glands. They will be in close proximity to hair follicles. Usually only portions of alveoli can be seen but are easily identified by their cells, which are pale with a frothy cytoplasm. Also examine **<u>slide 39</u>**, a section through the **infraorbital pouch** of the sheep. The sheep's infraorbital pouch is a cutaneous gland located just in front of the eye (see Dyce, 4th ed., p.369 for images), and serves as a territorial marker. The skin of this region contains large **sebaceous glands**, portions of which will be clearly evident throughout the section.

LAB 4 CONNECTIVE TISSUE

 # 52 - jejunum
 # 22 - mesentery (rat)
 # 13 - lip (cat)
 # 33 - planum nasolabiale (cow)
 # 80 - epididymis (boar)
 # 26 - lymph node
 # 3 - tendon
 # 17 - arteries and veins
 # 72 - lung (cow)
 # 74 - kidney
 # 2 - umbilical cord
 # 73 - kidney

1. **Loose (Areolar) Connective Tissue**. A good example of loose connective tissue (CT) can be found in the mucosal lining of the digestive tract, **slide 52**. The **mucosa** consists of three tissue layers: **epithelium, lamina propria, and muscularis mucosae.** (We will discuss these tissue layers in more detail in the second half of the course.) **The lamina propria is composed of loose connective tissue.** Recall from Lab 2 that connective tissue consists primarily of scattered cells and extracellular matrix or ECM. In loose connective tissue ECM is a loose network of fine fibers and the ground substance, composed mainly of glycoproteins and glycosaminoglycans, located between the scattered cells and the fibers. The viewable ECM in the lamina propria is comprised of collagen fibers. Find the lamina propria in slide 52, located just deep to the epithelium. Using your 40x objective, you may be able to identify specific cell types common to loose CT, e.g., **fibroblasts**, **eosinophils, lymphocytes, and plasma cells** (these cell types will be discussed in later labs). You should also note smooth muscle fibers, blood vessels, and lymphatic vessels (also to be discussed later).

Slide 22 is a whole mount of a piece of **mesentery**, and another good example of loose connective tissue (mesentery suspends the organs in the thoracic and abdominal cavities). It consists of loose CT sandwiched between two layers of **mesothelium**. Mesothelium is the name given to the epithelium that lines mesentery (like endothelium is the name given to the epithelium that lines vessels). Note the loose arrangement of the **collagenous** (thicker, pink) and **elastic fibers** (thinner). You should also be able to identify **fibroblasts** and **mast cells**. Many slides will also show blood vessels running through the mesentery. You may be able to identify red blood cells inside the vessel for confirmation, but do not mistake the blood vessel (which is usually larger and thicker) for a collagen fiber.

Examine **slide 13**, the cat lip. This slide was stained with toluidine blue which colors mast cell granules purple. Note the abundance of **mast cells** throughout the connective tissue.

Recall from Lab 2 that the superficial layer of the dermis, known as the papillary layer, is also made up of loose CT.

2. **Dense Irregular Connective Tissue**. On **slide 52**, note that between the mucosa layer and the thick outer layer of smooth muscle (known the **muscularis externa)**, is a layer called the **submucosa**. **The submucosa consists of dense**

irregular connective tissue. Examine the submucosa. Note that there are more thick collagen fibers than cells (hence the name dense), but that the collagen fibers are arranged in seemingly random orientations (hence the name "irregular"). The submucosa is surrounded on both sides by smooth muscle. Closer to the epithelium, the submucosa is bounded by a thin layer of smooth muscle called the muscularis mucosae. On the opposite side, the submucosa is bounded by a thick layer of smooth muscle known as the muscularis externa. Note: Your section may have a gap (an artifact) between the muscularis externa and the submucosa.

Be sure you can distinguish smooth muscle from collagenous fibers. Collagen is markedly eosinophilic (pink because it stains with eosin) and has a refractile quality. Smooth muscle appears dull in comparison and its eosinophilia is tarnished by a slight purplish haze. Also, collagen is an extracellular material while a smooth muscle fiber is a cell with a central nucleus. In this slide it is fairly easy to make these distinctions, but in other slides the differences are not as obvious. Learn them now, and later you will have an easier time.

The deeper, **reticular layer of the dermis** as seen in the **<u>planum nasolabiale</u>, <u>slide 33</u>**, provides an excellent example of **dense irregular CT**. Note the presence of large, coarse collagenous fibers. In contrast, the more superficial **papillary layer** of the dermis is formed from **loose CT**. It has finer fibers, a looser texture and generally more scattered cells than the reticular layer. Recall we examined this slide in Lab 2.

Examine the **connective tissue capsule** of the **<u>boar epididymis</u>, <u>slide 80</u>**. This is an example of a particularly compact dense irregular connective tissue. The fibers are woven together to form a tightly knit outer covering. Other organs, e.g., lymph nodes, also have this type of outer coat but it may not be as thick. Examine the **<u>lymph node</u>, <u>slide 26,</u>** to view this.

3. **<u>Dense Regular Connective Tissue</u>**. Examine a piece of **<u>tendon</u>, <u>slide 3</u>**. Note that unlike the collagen fibers viewed in submucosa of slide 52, or the collagen fibers viewed in reticular layer of the dermis in slide 33, the collagen fibers in slide 3 show a preferred orientation (hence the name "regular"). The parallel arrays of collagenous fibers are so dense that only the nuclei of the fibroblasts can be seen in between.

4. **<u>Elastic Tissue</u>**. This type of CT is found in the ligaments of the vertebral column and the suspensory ligament of the penis. You will view it macroscopically when you dissect out the nuchal ligament of the dog and horse. Fresh, unfixed elastic tissue has a yellow color in contrast to the white color of tendon (dense regular CT). Histologically, however, they are similar in appearance unless special stains such as Weigert's, resorcin fuchsin, or orcein are used to differentiate between them. Examine the demonstration slides. Weigert's stains elastic fibers purple or dark blue, while orcein colors them red. Elastic fibers can also be found in other places, e.g., **<u>walls of arteries</u>, <u>slide 17</u>**;

pulmonary alveolar septa, **slide 72**; and the vocal cords (no slide).

5. **Reticular Tissue**. Reticular tissue is characterized by an abundance of **reticular fibers**. These fibers cannot be distinguished from other collagenic fibers in H&E preparations. However, reticular fibers are **PAS positive** (PAS = **P**eriodic **A**cid-**S**chiff, a staining method used to detect structures with a high proportion of glycogen or carbohydrate macromolecules). Reticular fibers are also argyrophilic (i.e., contain cells that reduce silver solution to metallic silver, a staining method that is used to identify proteins, like collagen III, i.e. reticular fibers) and can be identified following processing by either of these procedures. **Slide 74** of the **kidney** has been stained using a silver procedure. Examine this slide and note how the reticular fibers have become blackened by the silver. What other organs are supported by a framework of reticular tissue?

6. **Mucous Tissue**. The CT of the umbilical cord is an example of mucous tissue. It has a jelly-like consistency and consists of an amorphous matrix containing collagenous fibers as well as a few elastic and reticular fibers. The cells present are mainly fibroblasts. Examine **slide 2** of the **umbilical cord**.

7. **Adipose Tissue**. This tissue consists of collections of **adipocytes** in **loose CT**. Adipocytes accumulate large deposits of lipids that are dissolved and extracted during the preparation of the slide, leaving behind a large empty vacuole surrounded by a thin rim of cytoplasm. Sometimes the flattened nucleus of the adipocyte may be seen. Examine **slide 73** for an example of adipose tissue.

LAB 5 CARTILAGE

 # 69 - trachea
 # 4 – elastic cartilage
 # 68 - epiglottis
 # 5 - fibrocartilage
 # 23 – bone marrow (optional)
 # 7 – bone, spongy: decalcified

There are three types of cartilage classified according to their extracellular components. These are hyaline, elastic, and fibrous cartilage.

1. **Hyaline Carti1age**. This is the most prevalent cartilage in vertebrates. Its name derives from the clear appearance of the matrix (Gk. *Hylos*, glass). In the fetus, hyaline cartilage forms most of the skeletal blueprint before it is replaced by bone and bone marrow through a process known as **endochondral ossification** (see below). In adults**,** hyaline cartilage persists as the nasal, laryngeal, tracheobronchial, and costal cartilage. Hyaline cartilage also is found at articular surface of synovial joints (e.g. knee, shoulder). This tissue has tremendous resilience and is uniquely suited for withstanding compressive forces.

Examine **slide 69** of the **trachea** for an example of **hyaline cartilage**. It has an avascular extracellular matrix (ECM) composed of thin collagenous fibers (type II collagen) embedded in an amorphous material consisting largely of sulfated proteoglycans (e.g. the large proteoglycan aggrecan, which contains aggregates of hyaluronic acid, and chondroitin sulfate and keratan sulfate). The sulfate groups on the chondroitin and keratan sulfate molecules strongly attract basic dyes and thus determine the basophilic staining characteristic of cartilage. Thus, if your slide is stained with H&E, the cartilage ECM will stain a very strong purple (which you should be able to see by simply holding the slide up to the light).

Most cartilages are sheathed by a **two-layered perichondrium**. The outermost layer of the perichondrium consists of dense irregular connective tissue (type I collagen); the cells (fibroblasts) in this layer are spindle-shaped. The inner layer is chondrogenic consisting of 2-3 layers of flattened, fibroblast-like chondrogenic cells. These cells have the capacity to differentiate into **chondroblasts** (young cartilage cells), which appear more rounded than the perichondrial cells. Chondroblasts secrete ECM and become trapped within it. They are then called **chondrocytes**. During tissue preparation, **chondrocytes** retract from the lacunar walls, often leaving gaping holes. This shrinkage is an artifact and does not exist in life. Sometimes pairs or clusters of chondrocytes are seen separated by a very narrow matrical septum. These are likely daughter cells of the same mitotic division, and are referred to as **isogenous groups**. The ECM immediately enveloping the chondrocytes is the **territorial matrix**, which is separated by a wide and paler **interterritorial matrix**.

2. **Elastic Cartilage**. The structure of elastic cartilage is similar to that of

hyaline cartilage except that the ECM contains abundant **elastic fibers**, which are synthesized by **chondrocytes** (in addition to type II collagen and sulfated proteoglycans). Elastic cartilage is found in the auricle of the external ear, a major portion of the epiglottis, and some of the laryngeal cartilages. The specialized matrix has remarkable flexibility and the ability to regain its original shape after deformation.

Examine **slide 4** (**external ear**) and **slide 68** (**epiglottis**). The **chondrocytes** in elastic cartilage are similar in appearance to those in hyaline cartilage, and are also surrounded by **territoria**l and **interterritorial matrices**; however, the ECM contains many **elastic fibers**. In H&E stained sections, these fibers appear as pale pink strands in a somewhat more basophilic background as can be seen in some slides of the epiglottis. In other preparations of the epiglottis, the fibers have been stained either purple with Weigert's (an elastic tissue stain), or red with orcein. Note the distribution of these fibers in relation to the chondrocytes and the surrounding **two-layered perichondrium**. Like hyaline cartilage, elastic cartilage is avascular.

3. **Fibrous Cartilage (fibrocartilage)**. Fibrous cartilage is generally avascular, and lacks a perichondrium. It is a hybrid tissue, with many characteristics of **dense connective tissue and hyaline cartilage**. Unlike hyaline cartilage, it is opaque, and its ECM contains both type I and type II collagen fibers, and a low concentration of proteoglycans. Fibrous cartilage has great tensile strength, and is usually located in transitional zones between hyaline cartilage and dense connective tissue. It is present in pubic symphyses, intervertebral discs, and at points where tendons insert into bones.

Most of the tissue on your preparation, **slide 5** (**intervertebral disc, horse**), is dense, irregular connective tissue. **Fibrous cartilage** is located on one side of the preparation and can be recognized by the slight basophilia in this region. **Chondrocytes** appear as small, somewhat isolated cells located in **lacunae**. They are usually arranged into short columns or rows (e.g. isogenous groups), separated by bundles of collagenous fibers and dense connective tissue.

Optional: Slide 23 (**mouse sternebra or vertebral column**): A few of your slides will contain fibrous cartilage in the intervertebral disc region of the sternebra. This is recognizable by chondrocytes with a slight basophilic ECM around them, arranged in a few rows, resembling beads on a string. Most of your preps, however, will either contain hyaline cartilage, or dense irregular connective tissue in this region.

Endochondral Ossification

Endochondral ossification (or **EO**, also known as intracartilaginous ossification) is one process by which the skeleton forms. During EO, skeletal “blueprints” made up of hyaline cartilage are replaced by bone and bone marrow. In vertebrates, most skeletal elements from the neck down (e.g. axial and appendicular skeleton), as well as certain cranial bones, form by this process. (Please note that the periosteal bone present in these elements, as well as many cranial bones, arise through a different mechanism known as **intramembranous ossification [IO]** discussed in the next lab on Bone). Both of these processes will be covered in detail in lecture.

Slide 7 shows a portion of a **developing long bone** from a rat fetus. Longitudinal growth of long bones depends upon the presence of **cartilaginous growth plates** (also known as epiphyseal plates or discs), which are found at outer tissue ends. During EO, chondrocytes within the growth plate undergo a series of maturation events including cell proliferation and hypertrophy. Blood vessels then invade the hypertrophic cartilage, and import a variety of cell types that cause degradation of hypertrophic cartilage, deposition of trabecular bone on top of hypertrophic cartilage cores, and establishment of a marrow cavity. You should be able to observe these events in your slide. **Slide 7** will contain either a growth plate or a maturing epiphyseal cartilage at the outer (bulbous) end of the long bone. The cartilage in these regions is divisible into five zones. These are: **1. Resting zone** (or “zone of reserve cartilage”); this zone is unaffected by EO, and is recognizable as being closest to the epiphysis and by containing randomly scattered chondrocytes; **2. Zone of proliferation** (or “zone of multiplication”); this zone is recognizable by the stacking of chondrocytes into vertical columns (isogenous groups); **3. Zone of hypertrophy** (this zone is recognized by the swelling and increase of cell size); **4. Zone of degradation** (or “zone of calcification and erosion” in certain species; this zone is represented by one terminal layer of hypertrophic chondrocytes that are facing the trabecular bone and marrow); and **5. zone of ossification** (recognized by trabecular bony spicules with hypertrophic cartilage cores). This zone should contain a large number of bone-resorbing cells or **osteoclasts**, which would be present on trabecular bone surfaces in resorption pits or **Howship’s lacunae**. Osteoclasts are derived from the monocytic lineage, and are multinucleate (anywhere from 2-40 nuclei).

You should be able to identify and understand the significance of each of these 5 sub-regions. Also, be sure to be able to identify the marrow.

Optional: Slide 23 (**mouse sternebra or vertebral column**): Try to locate the cartilaginous growth plate, which will only be a few cell layers thick, the trabecular bone (e.g. zone of ossification), and the marrow.

LAB 6 BONE

 # 8 – bone, compact: ground
 # 7 – bone, spongy: decalcified
 # 6 – bone, compact: decalcified
 # 11 – bone, membrane: decalcified
 # 23 – bone marrow section (optional)

Bone develops by replacement of a preexisting connective tissue. The two processes of bone formation or osteogenesis observed in the embryo are: **1. Endochondral ossification (EO)**, where bone and marrow replace a preexisting hyaline cartilage template or anlagen of future bone; and **2. Intramembranous ossification (IO)**, where bone is deposited directly within primitive connective tissue or mesenchyme. In both cases, a primary or immature bone is first laid down, and is then transformed ("remodeled") into mature bone. By these two processes, two types of bone are formed. **Cancellous** (also called **spongy** or **trabecular**) bone results from EO; this tissue is web-like and porous, is surrounded by bone marrow, and likely provides appropriate niches for blood cell development in the marrow cavity. **Compact** (also called **dense** or **cortical**) bone results from IO; this tissue is dense, and is designed for a supportive and weight-bearing role.

The bone extracellular matrix (ECM) consists primarily of type I collagen fibers, and an amorphous ground substance that contains small, non-sulfated proteoglycans. In addition, the ECM is mineralized, and thus is very hard. The calcium salts of the ECM are in the form of crystals called hydroxyapatites. These are embedded within and located on the surface of the type I collagen fibers. Bone cells or **osteocytes** are embedded within this mineralized ECM.

1. **<u>Compact bone</u>** is derived by IO, and is designed for its supportive and weight-bearing role. It consists of large amounts of calcified matrix and relatively little cellular material. It is found in such regions as the shaft of long bones, and many cranial bones. In this lab, the structure of compact bone is studied using slides of ground bone and tissue sections of bone.

The **<u>ground bone</u>** on **<u>slide 8</u>** has had all of the organic material removed. (You may have two sections on your slide; one is a cross section, and the other is a longitudinal section). To generate this slide, bone chips were hand ground with a fine abrasive to produce a translucent section. The mineralized matrix appears as light regions while cavities, once containing soft tissue, appear as dark regions. In a cross section, the basic unit of compact bone, the **osteon** (Haversian system), can be easily seen. This consists of a central **Haversian canal** (dark region) surrounded by concentric **lamellae** of bone ECM. Small, concentrically arrayed holes surrounding the Haversian canal represent **osteocyte lacunae**. If you focus up and down, tiny projections resembling spider webs radiate from the lacunae. These are **canaliculi**. Haversian canals, in life, contain blood vessels, nerves and loose connective tissue. They act as nutritive channels for bones. Materials do not readily diffuse through bone matrix. To facilitate diffusion and communication, osteocytes send out many fine cell processes known as filopodia to adjacent osteocytes. These processes are located in the canaliculi. The latter connect the Haversian canal to all osteocytes within the osteon. Haversian canals run parallel to the long axis of the bone. They are interconnected by transverse or oblique canals called **Volkmann canals**. Locate these canals in cross and if possible, in longitudinal sections of ground bone. **Interstitial lamellae** are located in the spaces between osteons. They represent remnants of older osteons that have been partially removed.

<u>Slide 6</u> and **<u>slide 23</u>** are **<u>longitudinal sections of bones</u>** that were decalcified by acid to facilitate sectioning. In **slide 6**, collagenous fibers are stained red. Note that individual fibers and fiber bundles cannot be seen. The cellular components, although somewhat distorted, remain. **Osteocyte**s can be seen in **lacunae** while blood vessels and connective tissue can be seen in **Haversian canals**. Osteons are not as readily apparent here. Spicules of **trabecular bone** can be seen radiating into the marrow cavity on **slide 23** (section of a sternebra from a mouse); while **compact bone** (whitish, poorly stained in our preps) is seen at the diaphyses. The **marrow cavity** is largely occupied by hematopoietic tissue, to be looked at in an upcoming lab.

2. **<u>Cancellous bone</u>** is derived by EO, and is arranged into a three dimensional web like network with the intervening spaces occupied by marrow. In the adult cancellous (trabecular) bone persists in the marrow cavities and at the ends of long bones (the epiphyses). Reexamine **<u>Slide 7</u>** of a **<u>developing long bone</u>** from a fetus. The diaphyseal region (shaft) is immature compact bone (compare to the hypocellular mature compact bone in **Slide 6**), while the cancellous bone is represented by the trabecular projections extending from the growth plate into the **marrow.**

<u>Intramembranous Ossification</u>

This term is applied when bone development occurs directly within or below a connective tissue membrane. Unlike the endochondral method, a cartilage model is not formed and then replaced by bone and marrow during the course of development. Most flat bones, e.g., frontal, parietal, dentary and maxilla, arise by the intramembranous method. Within the mesenchyme (connective tissue membrane) of the embryo where a flat bone is to appear, **osteoblasts** will secrete bone matrix in the form of spicules. These enlarge into trabeculae (slightly larger than spicules), which fuse together, forming a 3-D latticework as the bone grows and develops to its final size and form. A layer of bone-forming cells or **osteoblasts** is typically seen on the spicule surface. Often a layer of pale matrix is seen lining the spicules; this ECM is still uncalcified and is called **osteoid**. Examine **<u>slide 11</u>** for an example of **<u>developing membrane bone</u>**.

LAB 7 BLOOD

 # 9 or 99 – peripheral blood smear (dog or cat)
 # 1 – peripheral blood smear (horse)
 # C-35 – peripheral blood smear (parrot)
 # C-36 – peripheral blood smear (chicken or other species)

MAMMALS

1. **Slide 9** is a smear of **peripheral blood** from a **normal adult dog**, and **slide 99** is a smear of peripheral blood from a **normal adult cat**, stained with Giemsa. You should have at least one of these in your box. Examine the smear with your l0X objective. The purple nuclei of white blood cells are scattered among the more numerous pink stained red blood cells. Only the nuclei of the white blood cells are visible at this magnification since the cytoplasm is pale and not easily seen. Scan the slide with your 40x objective. Examine areas of the smear where red cells are evenly dispersed (monolayer), i.e., individual cells are easily discernible. Note the biconcave discoid shape of the red cell. Note the different types of white blood cells by the various shapes of their nuclei. Examine the individual white cells with your oil immersion objective. Remember that white blood cells will not always have a typical appearance if they have been distorted (smudged) in the smear preparation. You should be able to recognize the various different white blood cells as well as know their function.

Red Blood Cells (Erythrocytes)

Small, anucleate cells that are acidophilic and therefore appear orange to red using normal staining techniques. Their diameter varies with species: goats - 4.1 microns (the smallest), dogs - 7.0 microns (the largest). Their biconcave shape causes a central pallor.

White Blood Cells (Leukocytes)

Neutrophils. These cells normally constitute the majority of circulating leukocytes in dogs, cats, and horses. The nucleus contains condensed areas of **chromatin** and appears segmented, hence the name **polymorphonuclear**. The cytoplasm is pale gray with faintly staining granules.

Eosinophils. Few of these cells are present in the peripheral blood of normal animals. The nuclear characteristics are similar to the neutrophil but the cytoplasmic granules differ. Cytoplasmic granules are round, orange-pink and vary in size and number. The plasma membrane may be broken in some cells, and the granules will be dispersed around the nucleus of the cell.

Basophils. These cells are rarely found in mammalian blood. Like the neutrophil and eosinophil, the nucleus is segmented and contains areas of condensed chromatin, but the cytoplasmic granules characterize the cell. Basophil granules stain dark blue or purple and vary in size and number.

Lymphocytes. Lymphocytes are spherical cells that are usually smaller than neutrophils. The nucleus is round with coarse, clumped chromatin. There is a thin rim of blue cytoplasm; however the larger lymphocytes may have proportionately more cytoplasm. Lymphocytes normally constitute the majority of leukocytes in pigs and ruminants.

Monocytes. These cells are slightly larger than neutrophils and lymphocytes. The nuclear chromatin is looser than in a lymphocyte and stains lighter. The nucleus may be irregular or elongated; it frequently is kidney-shaped. Cytoplasmic vacuoles are often present and vary in size giving a foamy appearance to the blue-gray cytoplasm. Monocytes are often difficult to differentiate from large lymphocytes.

Platelets. Platelets are fragments of megakaryocyte cytoplasm and contain no nuclei. They may vary in size but are usually smaller than a red blood cell. They can be seen as faintly-stained, blue, granular structures. Sometimes they appear clumped.

2. Examine **slide 1**. This is a **blood smear** from a **horse**. Compare it to the dog blood smear. Note especially how the **eosinophils** of the horse differ from those of the dog.

BIRDS

3. **Slide C-35** is a peripheral blood smear from a **parrot**. **Slide C-36** is a **peripheral blood smear** from a **chicken**.(some may be from another avian species). The most obvious difference when compared to the dog is that the mature avian erythrocytes have nuclei. This is also the case with fish, amphibians and reptiles. In general, avian blood cells tend to be more fragile than mammalian cells and, therefore, are more variable in appearance. Also, there are usually more smudged cells present.

Erythrocyte. The mature red blood cell is an oval cell with an oval nucleus. The cell is larger than a normal mammalian red cell; its actual size will vary among species. Standard staining gives an acidophilic, orange-yellow color to the cytoplasm. As with mammals, this is due to the incorporation of hemoglobin. Therefore, less mature cells will have a more blue, basophilic, appearance. These younger cells are fairly common in the peripheral blood of normal birds.

Thrombocyte (Spindle Cell, Thigmocyte) - The shape and staining of these cells is very variable and can be affected by the presence of anticoagulant, the technique by which the smear is made, age of the sample, and the staining procedure. On a dried, stained smear the thrombocyte can range in appearance from a small, pale blue cell with a rounded nucleus, to a naked looking nucleus with only a thin rim of cytoplasm. No matter what the shape, general features are a dark, condensed nucleus with pale basophilic cytoplasm. The most identifiable characteristic, when present, are 1-4 dark magenta specific granules which are only found in thrombocytes. These granules contain 5-Hydroxytryptamine (5-HT, or serotonin), and break down when blood is shed.

This release of 5-HT causes the thrombocytes to clump, and sometimes these aggregates are seen in smears. The clumps of thrombocytes form a plug in the damaged vessel wall to stop the bleeding. In this way they act similarly to mammalian platelets, although they do not have identical properties. The origin of the thrombocyte is not definitely known. Two theories suggest that either they are part of the red cell lineage or they represent a completely separate line.

Heterophils. These cells are analogous to the typical mammalian neutrophil and very similar to the pseudoeosinophil of lagomorphs (rabbits and hares) and hystricomorph rodents (porcupine, capybara). Like thrombocytes they are present not only in birds, but in reptiles, amphibians and fish. The secondary granules of heterophils vary among species but they are most often described as being large and rod-shaped. They usually stain red to pink but may be gray in color and are scattered throughout the cell. The basophilic nucleus is lobulated.

Eosinophils. These cells are also very variable. Classically, their granules are described as being small and round. Eosinophils are usually less abundant than heterophils.

Basophils. These are similar in appearance to mammalian basophils, and in parrots may be as rare to find as in mammals.

Lymphocytes. Some divide this cell type into size categories small, medium and large, and use this system in assessing the health of an animal. In this particular lab we will concentrate on looking for typical cells. Lymphocytes have a large blue to magenta, eccentrically placed nucleus. The amount of dark blue cytoplasm is usually small in relation to the nucleus. Try to differentiate these cells from thrombocytes, with which they can be easily confused. Thrombocytes will typically have a denser nucleus and paler colored cytoplasm than lymphocytes.

Monocytes. These are similar to mammalian monocytes, they are larger than the other cells with irregularly shaped nuclei and cytoplasm. Commonly the cytoplasm is light blue with obvious vacuolization. Concentrate on finding typical monocytes and distinguishing them from typical lymphocytes and ignore the uncertain ones in the middle.

LAB 8 BONE MARROW

#18 – bone marrow smear (dog)
#24 – bone marrow smear (cat)
 # 25 - bone marrow smear (dog)
 # 23 - mouse sternum (embedded in plastic)
 # C-39 - bone marrow (duck)

MAMMALS

1. **Slide 25** is a smear of **bone marrow from a dog**. Examine the smear with the 10x objective. The **hematopoietic cells** vary in size and staining qualities. Some of the cells are clumped together and red blood cells can be seen among the bone marrow cells. Scan the smear with the 40x objective and note the variation in cell type. To distinguish the **red cell precursors, erythroid series**, from the **white cell precursors, granulocytic or myeloid series,** the oil immersion objective should be used. You may also have another bone marrow smear, **slide 24** in your slide box, **bone marrow from a cat**.

Slide 18 is a smear of **bone marrow from a dog**. If you had difficulty finding some cell types using slide 25, please try using this slide as an alternate.

There are more white cell precursors than red cell precursors, and mature cells predominate over the immature forms. In general, cells of the erythroid series have round nuclei with dense, darkly stained, clumped chromatin and basophilic cytoplasm. As the cell matures, the cytoplasm becomes hemoglobinized, i.e., more acidophilic and less basophilic, while the nucleus condenses, becomes smaller and is eventually extruded. Cells of the granulocytic series have different nuclear and cytoplasmic characteristics. The cell nucleus is lighter purple and has a finer chromatin pattern. The nuclear shape changes as the cell matures becoming successively flattened, eccentric, elongated and lobulated. The cytoplasm is initially basophilic and granular, but the intensity of basophilia decreases with maturation. Note the different stages of erythroid and granulocytic differentiation.

Erythroid series

Rubriblast and **Prorubricyte (Basophilic Erythroblast):** These are large cells with a darkly staining, round, centrally-located nucleus containing coarse and clumped chromatin; the cytoplasm is very basophilic (navy blue) and smooth. The rubriblast differs from the prorubricyte in that a prominent nucleolus will be present – a nucleolus appears as a different colored spot (typically blue) within the more purple staining nucleus.

Rubricyte (Polychromatophilic Erythroblast): This cell is smaller than the prorubricyte; the nucleus contains denser chromatin and it occupies a smaller portion of the cell. Hemoglobin synthesis imparts a pink color, here and there, to the cytoplasm providing an overall mottled or lavender appearance (polychromasia).

Metarubricyte or Normoblast (Orthochromatic Erythroblast): The cell is smaller than a rubricyte. Its nucleus contains very condensed (almost pyknotic) chromatin and is darkly stained. Its cytoplasm is frequently pink or at least lighter in color due to the increased hemoglobin content.

Polychromatophil (Reticulocyte): Immature, anucleate red blood cell. Cytoplasm is more lavender than a mature erythrocyte because of a decreased hemoglobin content. A reticulocyte count is used to assess regenerative response in anemic patients. **(You are not required to find this cell)**

Erythrocyte: Mature red blood cell.

Myeloid series

Myeloblast: This is a large cell with a round to oval nucleus and finely dispersed chromatin - euchromatic. Nucleoli may be present. The cytoplasm is basophilic and grainy. **(This cell is very rare and is not a required "find".)**

Progranulocyte (Promyelocyte): In this cell, the nuclear chromatin is beginning to clump. Magenta or purple (azurophilic) granules are visible in the basophilic cytoplasm. Neutrophils, basophils, and eosinophils are indistinguishable up to and including this stage of development.

Myelocyte. The myelocyte is smaller than the progranulocyte. Its nucleus is usually oval and eccentrically situated. The nuclear chromatin is coarser. Specific granules of the neutrophilic, basophilic, and eosinophilic series now develop in the cytoplasm.

Metamyelocyte. The nucleus begins to indent to form lobes (sausage-shaped) and the chromatin becomes denser. The cytoplasm is paler blue.

Band Cells and Mature Segmented Granulocytes. As the nucleus matures, it continues to indent to form a band or rod shape. The nucleus eventually becomes segmented in appearance in the mature neutrophil, basophil, and eosinophil. The cytoplasm is pink and specific granules may be seen.

In addition to the above cells the following cells may be seen in bone marrow smears: Megakaryocytes (easiest from low magnification), Osteoclasts (rare), Osteoblasts (rare), Plasma Cells (few), Cells in Mitosis (few), Lymphocytes (few), Macrophages (few) and Adipocytes.

2. Examine **slide 23**. This is a section of the **sternum of a mature mouse** and shows the contained bone marrow. The tissue was embedded in plastic and stained with Giemsa. Sections such as this permit the evaluation of the cellularity and architecture of marrow which is not possible with a bone marrow smear (cytology). Note that the marrow is packed with hematopoietic cells. The erythroid precursor cells stain darker than the granulocytic cells and they are less numerous. Try to identify different cell types. Note that **megakaryocytes** frequently are positioned next to vascular sinuses. Why might this be an asset given their function?

BIRDS (AVES)

3. **Slide C-39** contains a number of sections through **bone marrow of the duck**. A major difference here is in the **organization of the hematopoietic tissue**. As in mammals, myelopoiesis occurs in the space outside of the vessels of the marrow. However, in birds erythropoiesis takes place within the blood vessels of the marrow. The earliest red blood cell precursors are in close association with the endothelium of the vessel wall. As the cells mature, they move out towards the center. Try to trace the endothelial border and identify developing erythrocytes.

Aside from the fact that avian red blood cells do not extrude their nuclei, bone marrow smears from birds can be interpreted in the same manner as for mammals. The immature forms can be named similarly.

LAB 9 LYMPHATIC TISSUE

 # 53 - ileum
 # 29 - tonsil (horse)
 # 30 - tonsil (dog)
 # 26 - lymph node
 # 28 - hemal node
 # 32 - spleen
 # 31 - thymus
 # C-40 - bursa of Fabricius (avian)
 # C-28 – spleen (avian)

1. **Lymphatic Nodules**. **Slide 53** is a section of the **ileum**. Clustered lymphatic nodules composing a **Peyer's patch** are present in the lamina propria and/or submucosa at one side. Collections of **lymphocytes** in H&E stained tissue sections are recognized at low magnifications as deeply basophilic areas. This appearance is due to the presence of many small lymphocytes that have deeply stained nuclei and very little cytoplasm. Some nodules have central pale areas due to the presence of lymphocytes with paler nuclei and larger amounts of cytoplasm; these areas are **germinal centers**. The rim of small lymphocytes surrounding the germinal center is called the **corona** or **marginal zone** of the nodule. The nodules in a Peyer's patch are partly **encapsulated by dense irregular connective tissue.**

2. **Tonsils, slides 29 and 30**. Tonsils lie beneath the **stratified squamous epithelium** that lines the oral cavity. The surface over the tonsil may be smooth or it may form deep invaginations called **crypts**. Basically, tonsils are collections of **nodules** and diffuse **lymphatic tissue** in the subepithelial connective tissue. **Slide 29** is a section of **tonsil from a horse**. Several crypts are present. The connection of some of the crypts to the surface may not be visible and these appear as isolated slit-like spaces lined by stratified squamous epithelium. The epithelium over the tonsils is infiltrated in places by **lymphocytes**. Notice that the tonsil is organized into nodules and internodular areas. Some **mucous salivary glands** are seen in this slide.

Slide 30 is a section of the **palatine tonsil of a dog**. Notice that there are no crypts within the tonsil. The entire tonsil lies within a fossa, and is covered in part by a semilunar fold. **Salivary glands (mixed)** and some **skeletal muscle** are included in this section.

3. **Lymph Nodes**. **Slide 26** contains sections of **lymph nodes**. Using low magnification, note the architectural features characteristic of lymph nodes including the **capsule and trabeculae** (extensions of the **dense connective tissue** of the capsule that dip into the interior), **cortex and medulla**, **subcapsular sinus**, and **medullary cords and sinuses**. The cortex is composed of **nodules** (**B cell areas**) and the internodular and **deep cortical lymphatic tissue** (**T cell areas**). Many nodules have **germinal centers**.

The medulla has **medullary cords**, densely cellular extensions of cortical tissue, and the more loosely cellular **medullary sinuses**. Medullary sinuses are crossed by many fine strands of pink cytoplasm representing the branches of reticular cells - the fibroblastic cells that form the meshwork in most lymphatic tissue. Depending on how good your section is, you **may at 40x and 100x** be able to identify some individual cells in the medulla as lymphocytes, plasma cells, macrophages and reticular cells. The identity of a cell as a macrophage is established by phagocytized particles in its cytoplasm. Macrophages often contain hemosiderin, a golden-brown pigment resulting from the breakdown of red blood cells.

4. **Hemal Nodes**. **Slide 28** is a section of a hemal node. Hemal nodes are peculiar lymphatic organs found in some species, especially in ruminants. They are similar in architecture to lymph nodes but are supplied with blood vessels. They lack lymphatic vessels. They are **encapsulated** and have a **subcapsular sinus**. **Nodular and diffuse lymphatic tissue** is surrounded by **blood-filled** communicating **sinuses** in a meshwork of cells that are probably **smooth muscle cells**.

5. **Spleen**. **Slide 32** contains two sections of cat spleen. One is relaxed and filled with blood (congested) and the other is contracted and contains much less blood. With the unaided eye, you can distinguish the areas of **white pulp** and **red pulp**. White pulp is blue/purple because it is composed of **lymphocytes** that take up hematoxylin. Using low magnification, observe the **capsule** and **trabeculae** and the **blood vessels** found in the trabeculae. Trabecular arteries have a distinct wall, whereas trabecular veins are endothelial-line tunnels within the trabecular substance. In the horse, cat and dog, the capsule and trabeculae are largely composed of **smooth muscle cells** which can relax and allow the spleen to fill with blood and contract and expel the stored blood back into the circulation. Compare the volume of red blood cells in the relaxed and contracted spleens to appreciate this blood storage capacity. Observe that areas of white pulp are oriented around **central arteries**, which are often not centrally located, in spite of the name. White pulp consists of the sheaths of **T lymphocytes** that surround the central arteries, which are known as **periarterial lymphatic sheaths, or PALS**, and the **nodules** of lighter staining **B lymphocytes** that are often embedded in the PALS. The red pulp surrounds the white pulp; the boundary between the two is the marginal zone. Look for **periarterial macrophage sheaths (ellipsoids)** in the marginal zone or red pulp. These sheaths of macrophages form large pale nodules around red pulp arterioles that may be confused with cross sections of trabeculae. Trabecular smooth muscle cells are pinker, have distinct cell borders and their nuclei are uniformly shaped and regularly spaced. Macrophage nuclei are larger and more irregularly shaped and spaced, the pale cytoplasm looks somewhat foamy and cell borders are not visible. Can you identify any of the cell types that are visible in the red pulp using 40x or 100x?

6. **Thymus, slide 31**. Observe that the thymus is surrounded by a **thin**

capsule of connective tissue from which extend **septa** that divide the gland into irregular **lobules**. Each lobule has an outer more darkly stained area containing small lymphocytes called the **cortex** and an inner **medulla** containing fewer and larger lymphocytes. Lobules are not totally separated from each other; the medulla of one is frequently seen to be continuous with that of others. Note that there are no nodules in the thymus as in other lymphatic tissue. What might be the explanation for this? Note the presence of pink laminated whorls of epithelial reticular cells in the medulla. These are called **Hassal's (thymic) corpuscles** and are highly diagnostic of the thymus.

7. **<u>Bursa of Fabricius, slide C-40</u>**. The bursa is a saclike, oval, dorsal diverticulum of the wall of the proctodeum, a part of the bird's cloaca. The lumen is lined by **pseudostratified columnar epithelium** under which lies a thick layer of **lymphatic tissue**. This tissue is divided into polyhedral **lobules (follicles**) by **connective tissue septa**. Each lobule has a **cortex and medulla**. The epithelium and underlying lymphatic tissue form folds that extend into the lumen of the bursa. As in the thymus, the meshwork supporting the lymphocytes in the bursa is formed by **epithelial cells**. There is a **capillary** which separates the **medulla from the cortex**. It can be identified by the nucleated red blood cells within it.

Birds do not have lymph nodes but lymphatic nodules are scattered throughout and are especially frequent beneath the epithelium of the digestive and respiratory tracts. Birds do have a thymus and spleen. The thymus is similar to that in mammals but the spleen is different. **<u>Slide C-28</u>** is a section of **<u>avian spleen</u>**. The **distinction between red and white pulp** is not as clear as in mammalian spleens. White pulp is seen as irregularly shaped collections of lymphocytes in which there are several small arteries each bearing a small pink collar representing a periarterial reticular sheath. Red pulp is very cellular (reticular cells, macrophages, lymphocytes and red blood cells); venous sinuses are also present in the red pulp. Several large arteries and veins can be seen but trabeculae are not present.

LAB 10 MUSCLE

 # 12 - muscle types
 # 37 - tongue
 # 38 – tongue
 # 14 - cardiac muscle (cat & goat)
#15 cardiac muscle (horse)
 # 46 - stomach

1. **<u>Skeletal Muscle</u>**. Use **<u>slides 12, 37 and 38</u>**. Slide 12 has examples of each of the three muscle types: **skeletal, cardiac and smooth**. On most slides skeletal and cardiac muscle have been stained with phosphotungstic acid in order to emphasize the **banding pattern**. These sections were then counterstained with eosin. Use your 40x objective to observe the banding pattern of skeletal muscle. Slides 37 and 38 provide better examples of skeletal muscle cut in various orientations. Skeletal muscle cells are **multinucleate.** Where are the nuclei located within the cell? Be able to identify **myofibrils** in cross sections of muscle cells.

2. **<u>Cardiac Muscle</u>**. Use **<u>slides 14, 15, and l2</u>**. Select areas where muscle cells are seen in longitudinal section and where the striations are well stained. Compare the striations with those of skeletal muscle. Are they the same? Note that **cardiac muscle cells branch** and that the cells have a **single, centrally located nucleus**. Can you find areas on your preparation where the muscle cells have been cut transversely? Be able to recognize **intercalated discs**. These can be seen in slides 14 and 15, or wherever the muscle cells have been sectioned longitudinally.

3. **<u>Smooth Muscle</u>**. Using **<u>slides 46 and 12</u>**, find areas of the sections where muscle cells have been cut longitudinally and transversely. Each smooth muscle cell is spindle-shaped and contains one **centrally located nucleus**. Can you distinguish cell boundaries in both longitudinal and transverse sections through smooth muscle cells?

LAB 11 BLOOD VESSELS AND NERVES

 # 37 - tongue
 # 30 - tonsil
 # 51 - pylorus and duodenum
 # 80 - epididymis
 # 17 - arteries and veins
 # C-33 - arteries and veins
 # 19 - aorta and vena cava
 # 20 - myelinated nerve
 # 21 - spinal cord (sheep)
 # 63 - ureter and ductus deferens

1. **Capillaries**. Using the **tongue** of the cat, **slide 37**, locate areas of **skeletal muscle** cut **longitudinally**. The **capillaries** may be found between the muscle fibers (cells). Search carefully until you find a longitudinal section of a capillary showing one or more endothelial nuclei. True capillaries are only large enough to permit the passage of one erythrocyte at a time and sometimes the erythrocytes can be seen in the capillaries lined up in single file. Study the characteristics of the endothelial cell nuclei carefully and learn to recognize them. Study also the appearance of **capillaries in cross section**. The tiny capillaries may be seen between the fibers in areas where the **muscle** is cut **in cross section**. Only occasionally will the capillaries be sectioned in the plane of a nucleus so that a nucleus is not often seen in capillaries cut in cross section.

2. **Small Arteries and Veins**. Use **slides 30, 51 and 80**. In these slides of the **tonsil**, **pylorus-duodenum** and **epididymis**, numerous **small arteries and veins**, cut in various planes, will be found. Small arteries can be defined as having up to 8 layers of smooth muscle in the wall. Arterioles, the smallest of the small arteries, have about 1 or 2 smooth muscle cells in their wall. Find a small artery and vein (they usually lie side by side) that have been cut transversely, i.e., in cross section. You will find these vessels by searching in the connective tissue. The vein will have a few collagenous fibers supporting its wall, whereas the artery will have a few smooth muscle cells around it. Often small fasciculi of nerve fibers accompany the blood vessels. Can you identify any?

3. **Medium-sized Arteries and Veins**. Use **slides 17 and C-33**. These sections contain many **small and medium-sized blood vessels**. Study the structure of arteries and veins of various sizes. Identify the **tunica intima** (an endothelial lining)**, tunica media** (usually made of smooth muscle) **and tunica adventitia** (of connective tissue) in both vessels. The tunica media of an artery tends to be the thickest and most well defined layer. In a vein the tunica adventitia tends to be the thickest. Medium arteries tend to have an internal elastic membrane. In most slide boxes slide 17 is stained by the Weigert's elastic tissue method and counterstained with hematoxylin.

4. **Large Artery and Vein**. On **slide 19** there are two sections of large blood vessels stained with a specific elastic tissue stain. The larger vessel with the

thicker wall is the **aortic arch**, and the smaller is the **precava** of a dog. Study both and note the differences between these and the smaller vessels. Note that the **media** is well developed and possesses numerous concentrically arranged elastic membranes in the aorta, but is a thin layer in the precava. The **adventitia**, however, is relatively undeveloped and nonmuscular in the aorta, but is well developed in the precava. Close to the latter's entrance into the heart, the adventitia contains cardiac muscle; elsewhere it may contain longitudinally arranged bundles of smooth muscle.

5. **Nerves**. Examine **slide 20** of a cross section of a **myelinated nerve** from a pig. Masson's trichrome stain was used to prepare this slide. Accordingly, **collagenous fibers** will appear blue-green and **smooth muscle** will be red. Note that the nerve consists of numerous **fascicles (bundles) of myelinated axons.** Each fascicle is surrounded by a layer of **dense connective tissue** called the **perineurium**. Wisps of connective tissue (**endoneurium**) extend inward from the perineurium to surround individual myelinated axons. Spaces appearing between the perineurium and axons are artifacts. A **dense connective tissue** layer called the **epineurium** forms the outermost wrapping of the nerve. The epineurium extends into the interior of the nerve as a fibrous network, the **interfascicular connective tissue**, surrounding the fascicles. What purpose is served by the connective tissue wrappings?

Examine **slide 21 (sheep spinal cord)**. This slide was stained with H&E. **Myelinated axons** are blue in H&E preparations. Observe the axons in sections through nerves located adjacent to the spinal cord. Many of the axons will appear shrunken and distorted. This is an artifact. Also observe the numerous **multipolar neurons** in the **gray matter** of the spinal cord. Many of these neurons are quite large. All of them have prominent nuclei.

Reexamine **slide 20** and note the presence of numerous blood vessels of various sizes. You should try to identify them. Also, note deposits of adipose tissue surrounding and within the nerve.

Examine **slide 63** which contains a section through the **ductus deferens** of the pig. There are sections through **unmyelinated nerves** present in this preparation. Compare these with the myelinated fibers studied previously. How do they differ from myelinated fibers?

LAB 12 ENDOCRINE SYSTEM

 # 94 - pituitary gland
 # 96 - pituitary gland
 # 95 - thyroid
 # 97 - parathyroid
 # 98 - adrenal gland
 # C-38 - adrenal gland (avian)

Slide 94 is a section of **pituitary gland** from the horse. It is triply stained with alcian blue, orange G, and Schiff's reagent. Study the section with the 4x objective and note that the **pars nervosa** (central portion) is stained blue. The **pars intermedia** (pink) enwraps most of the pars nervosa. The **pars anterior (distalis)**, orange and very vascular, constitutes the major portion of the section.

Study the pars distalis first. Note the many **sinusoids containing erythrocytes** (bright orange). Interspersed among the sinusoids you will see clusters of different cells. **Chromophils** are those cells possessing an affinity for certain dyes. Those with light orange cytoplasm are **acidophils**; those with blue or red cytoplasmic granules are **basophils**. **Chromophobes** are small, rounded or polygonal cells containing relatively little cytoplasm. They lack an affinity for the usual dyes used in histology and exhibit no visible granules when observed by light microscopy. Since the nucleus-to-cytoplasm ratio is high in chromophobes, clusters of them appear as groups of relatively tightly packed nuclei amongst the chromophils.

Various experimental procedures have established that growth hormone and prolactin are derived from the cells stained with orange G on this slide - acidophils. Follicle stimulating hormone (FSH), luteinizing hormone (LH), and thyroid stimulating hormone (TSH) are glycoproteins and are produced by basophils. These cells will be stained either red (PAS positive because of the large amount of glycoprotein) or blue (alcian blue). Chromophobes are considered to be degranulated acidophils or basophils. They may represent a source of adrenocorticotropic hormone (ACTH), or corticotropin.

Observe the cytological characteristics of the pars intermedia and pars nervosa. In the latter, note the numerous **capillaries** and the pale areas which are composed of **unmyelinated axons** from the nerve cells of supraoptic and paraventricular nuclei of the hypothalamus. **Vesicles (follicles)** containing colloid may be seen in the pars intermedia.

Slide 96 is a section of the **pituitary of the cow**. It is stained with H&E. Compare it with the preceding slide with regard to location and proportions of the **pars distalis, intermedia and nervosa**. Note that eosin stains the granules of the acidophils in the pars distalis a bright pink. Basophils accumulate some hematoxylin and tend to be larger than the acidophils. In the cow's pituitary, the lumen of **Rathke's pouch** persists and is evident on this section as a cleft between the **pars distalis and pars intermedia**. Note that the pars intermedia partially enwraps the pars nervosa and contains mostly basophils. In the pars nervosa, erythrocytes can be seen in the numerous capillaries. Also note the numerous small, round or oval nuclei that belong to cells called **pituicytes** which are neuroglia cells. H&E processing does not stain the cytoplasm of these cells. They are thought to have a supportive function only. Neurosecretory granules are stained a light pink and are not differentiated from the neuropil (the feltwork formed from axons and dendrites of unmyelinated neurons and the glial cell processes of this part of the pituitary).

The **thyroid, slide 95**, is surrounded by a thin **connective tissue capsule**. **Trabeculae** extend from the capsule into the parenchyma consisting of numerous follicles lined by a simple epithelium of low cuboidal or squamous follicle cells. When the gland is stimulated by TSH the cells become cuboidal or columnar. This section of the thyroid illustrates the appearance of the organ when a moderate to fair amount of **colloid** is stored. In this condition, some of the follicles are distended with colloid but a few contain very little. How would you explain the solid masses of epithelial cells evident in this section? Are **parafollicular cells** present in your preparation? Some of the slides may have portions of one or two other organs included. If yours does, can you identify the organs? Some slides may also have a heavy lymphocyte infiltration – this is normal in the aging thyroid.

Slide 97 contains a section of the **parathyroid gland**. Note the delicate **capsule** and **cord-like arrangement of the cells**. With the 40x objective, note how the cells are arranged around and along the capillaries. No oxyphilic cells are present. All of the cells are chief cells (also known as principal cells).

Slide 98 is a section of the **adrenal gland** of a horse. Observe the **capsule, cortex**, **medulla**, and **numerous sinusoids** (present throughout the organ). Three distinct zones of the cortex may be differentiated: **zona multiformis** (outermost), **zona fasciculata** (middle), **zona reticularis** (innermost). Study the cells in the various zones of the cortex and the medulla with the 40x and 100x objectives.

Cells of the zona multiformis (also called zona glomerulosa) are columnar. They are arranged in arc-like formations with the convexity nearest the capsule. The zona fasciculata consists of cuboidal or columnar cells arranged in the form of radial cords. Numerous **vacuoles**, which once contained **lipid**, can be seen in the fasciculata cells. The zona reticularis consists of anastomosing cords of cells. They contain fewer lipid vacuoles than those of the zona fasciculata.

Note the granular cytoplasm of the cells of the medulla. Sections of the tortuous **central vein** of the medulla may be visible. In some slides, **nerve cells** (individual cells or clusters of cells) may be found scattered through the medulla. These are ganglion cells of the autonomic nervous system. Because the cells of the medulla and cortex interdigitate, projections of the zona reticularis may be seen amongst the chromaffin cells.

Slide C-38 is a section of avian **adrenal gland**. Note that there is a **gross lack of organization** when compared to the mammalian adrenal. **Cords of cortical cells** (pink) are seen to be indiscriminately mixed with **cords of medullary cells** (purple). A rich capillary or arteriole/venule supply is evident, and at one pole of the organ a ganglion may be present in some slides.

LAB 13 MALE REPRODUCTIVE SYSTEM

 # 77 - testis (rat)
 # 79 - testis (boar / stallion)
 # 80 - epididymis (boar)
 # 78 - ductus deferens (dog)
 # 63 - ductus deferens (pig)
 # 81 - prostate gland
 # 82 - prostatic urethra (dog)
 # 83 - penile urethra (horse)

Examine the **rat testis, slide 77**. The testes are **compound tubular glands**. They are invested by a **thick capsule of dense irregular connective tissue**, the **tunica albuginea**. The latter is covered by a **peritoneum**, the visceral layer of the tunica vaginalis. It is composed of a mesothelium and underlying connective tissue that blends with that of the tunica albuginea. Observe the **seminiferous tubules** and note that there are oblique and cross sections of tubules which are not all in the same phase of activity. Study several tubules and note the following cells: **a. spermatogonia, b. primary spermatocytes, c. spermatids (early and late), d. spermatozoa (mature sperm cell), and e. Sertoli cells**. It is rare to observe secondary spermatocytes because this stage of division occurs very rapidly.

Now examine the **boar testis, slide 79** (note: some slides may be stallion testis and not boar. The stallion testis will have a muscle tissue layer in the outer capsule). Observe the compact clusters of **interstitial cells (of Leydig)**. These cells, which produce testosterone, are more numerous in the boar than in any other mammal. Observe the **dense connective tissue capsule** of the testis at one edge of the section.

Examine the **epididymis, slide 80**. The duct of the epididymis is one long, highly coiled tube. Therefore, a histologic section cuts through it many times. It is lined by a **pseudostratified epithelium with stereocilia**. Connective tissue and smooth muscle can be seen in its wall. Note the presence of a **thick collagenous capsule and supporting septa**.

Examine the **ductus deferens (vas deferens)** of the dog, **slide 78**. Note the **heavy muscular layer**. The inner portion is circular while the outer is longitudinal. The **epithelium is pseudostratified**, although toward the end of the duct it may be simple columnar.

Examine **slide 63, pig ductus deferens**. This slide also contains a section through the **ureter**; compare the latter with the ureter of the horse (slide 75), which we will examine in a later lab.

Examine the **prostate gland, slide 81**. Note the **fibromuscular capsule**, the broad prominent **trabeculae**, and the well defined **lobulation**. Note, also, the very irregular **secretory tubules** with numerous infoldings and the

pseudostratified columnar epithelium containing numerous secretory granules. The prostate is a **compound tubuloacinar gland** whose secretory cells are mainly of the **serous type**.

Examine the **prostatic urethra** from a young dog, **slide 82**. Note the following: 1. **the type of epithelial lining**, 2. **the cellular lamina propria**, 3. **the surrounding lobules** of prostatic tissue which are in an undeveloped and inactive state, and 4. the presence of **smooth muscle bundles** in the **capsule and septa**.

Examine the **penile urethra from the horse, slide 83**, and note the following: 1. the **type of epithelium,** which may be either stratified cuboidal or transitional; 2. the **lamina propria with lymphoid tissue**; 3. the layer of **erectile tissue**, consisting of spaces lined by endothelium (cavernous veins) in a fibromuscular stroma; this is the corpus spongiosum (also known as, in some texts, the corpus cavernosum urethra); and 4. **striated muscle**.

LAB 14 FEMALE REPRODUCTIVE SYSTEM I

 # 85 - ovary (cat)
 # 84 - corpus luteum (dog)
 # 86 - corpus luteum (cow)
 # C-22 - ovary (avian)
 # 87 - oviduct

Examine **slide 85, cat ovary**. Note: **cortex, medulla, cuboidal germinal epithelium** (not present in most slides)**, tunica albuginea** (thick connective tissue layer immediately below the germinal epithelium)**, primordial follicles, primary follicles (early ones with a single layer of cuboidal, follicle cells as well as later ones with multiple layers - multilaminar follicles), older developing follicles - secondary and tertiary (Graafian) and atretic follicles** (atretic follicles are those which are degenerating. Follicles can become atretic at any stage of development)**. Identity the following: oocyte, zona pellucida, membrana granulosa, follicular antrum, cumulus oophorus, corona radiata and follicular theca.**

Slides 84 (dog) and **86 (cow)** are of the **corpus luteum** of the dog and cow respectively. Note the proportional size of the **corpus luteum** in relation to the ovary as a whole. Note the characteristics of the **luteal cells**. In slide 84, **cords of epithelioid cells (interstitial gland cells)** may be found in the **stroma**. The **epithelioid cells** are derived from the theca interna of atretic, antral follicles or from the granulosa cells of atretic, preantral follicles.

Slide C-22 is of an **avian ovary**. Only one ovary (the left) persists as a functional organ in the hen. It exhibits a number of basic differences from the mammalian ovary. Note that a **cortex and medulla** can be distinguished and that the medulla is very vascular. On the surface there is a **germinal epithelium**. The **tunica albuginea, composed of dense connective tissue**, lies beneath the epithelium. A **stroma** of loose connective tissue containing developing follicles lies beneath the tunica albuginea. In certain areas **primary follicles** may be seen. At one edge of the section there may be one or more **ganglia**.

Study the structure of the ovarian follicles and note that the **ovum (primary oocyte) accumulates yolk** until it reaches a large size. **No antrum** develops for this reason. A **membrana granulosa** surrounds the oocyte. It is a layer of simple cuboidal cells in the smallest and largest follicles and is a pseudostratified layer in follicles of intermediate size. **Vacuolar cells** and **interstitial (luteal) cells** will be visible in your preparation. Numerous **fat vacuoles** occur throughout the cytoplasm of the vacuolar cells and their **nuclei are pyknotic** (small and condensed). Collections of these cells, in the cortex, may represent regressing postovulatory follicles. The pale, interstitial cells occur in groups in the compact connective tissue of the theca externa of the follicle. They may also be found, in groups, in the cortical stroma and medulla. Interstitial cells are thought to be the source of both estrogens and androgens. Despite early references to the presence of corpora lutea in the bird ovary, it is now thought that corpora lutea are not present and that progesterone is produced by cells of the membrane granulosa, the interstitial cells or possibly both.

Atretic follicles may be present in your preparation. In the most common type of atretic follicle, cells of the membrana granulosa proliferate, forming a number of irregular layers around the oocyte. The oocyte becomes smaller and is eventually replaced by granulosa cells. Scar tissue ultimately replaces the granulosa cells. In older birds, the oocyte becomes surrounded by hyperplastic and hypertrophied interstitial cells during atresia. Both the oocyte and the membrana granulosa eventually degenerate.

Examine the **oviduct of the cow, slide 87**. Note the **mucosa, muscularis and serosa**. The **folding** of the mucosa is characteristic of the cranial third but diminishes progressively caudally. The epithelium in this section is pseudostratified. Some cells possess cilia while others are secretory and lack cilia. The two types of cells may be distinguished using the oil immersion lens.

LAB 15 FEMALE REPRODUCTIVE SYSTEM II

 # C-23 – infundibulum (avian)
 # C-24 – infundibulum (avian)
 # C-25 – magnum (avian)
 # C-26 – uterus (avian)
 # C-27 – vagina (avian)
 # 88 - resting uterus
 # 89 - uterus in estrus

 # 91 -mammary gland (inactive)
 # 90 - mammary gland (active)
 # 92 - mammary gland (inactive)
 # 93 - teat

The **oviduct** of the laying hen is about 65 cm long and capable of great dilation. The tube is usually divided into five segments which, from cranial to caudal, are: **infundibulum, magnum,** isthmus, **uterus, and vagina**.

The oviduct synthesizes those coats, other than part of the perivitelline membrane, which surround the ovum in the hard-shelled egg. The magnum forms the albumen or white of the egg. The isthmus forms the keratinous shell membranes and the uterus forms the calcareous shell, pigment and cuticle. Chalaza formation has been attributed to the infundibulum, although the degree of its involvement is uncertain. The chalazae are thought to stabilize the position of the yolk within the albumen. The uterus functions to produce the outer shell, and is sometimes referred to as the shell gland. The vagina does not contribute to the formation of the egg, but its sperm-host glands serve as preferential storage organs for sperm, from which sperm are released over a period of a week or more, following normal copulation.

Examine the **infundibulum, slides C-23 and C-24**. The open end of the infundibulum is shaped like a funnel. The narrow end of the funnel is the neck of the infundibulum. A **mucosa, muscularis and serosa** are present. In the funnel, slide C-23, the epithelium is simple, ciliated columnar and the muscularis is comprised of scattered bundles of smooth muscle. In the neck of the infundibulum, the mucosa is thrown into primary folds and on the surface of these folds, are secondary and tertiary folds - see slide C-24. The epithelium is ciliated pseudostratified columnar except at the bases of the secondary folds where it consists of nonciliated, columnar, secretory cells. The muscularis is arranged into inner circular and outer longitudinal layers in the neck. The serosa of the infundibulum consists of mesothelium and underlying connective tissue.

Examine the **magnum, slide C-25**. Note the well developed **mucosal folds** (a delicate submucosa forms the core of each fold) and pseudostratified epithelium. The latter consists of ciliated columnar and secretory (goblet) cells. The nuclei of the secretory cells are rounded and lie closer to the basement membrane than the nuclei of the ciliated cells, hence the designation pseudostratified. The **muscularis** is better developed than in the infundibulum.

The **lamina propria** of the magnum is abundantly filled with **compound tubular glands**, which form the albumen of the egg. A **serosa** covers the external surface.

There is no slide of the isthmus. The glands of the isthmus produce the shell membranes.

Slide C-26 is of the shell gland (uterus). The uterus is an expanded portion of the oviduct. Its walls are not as thick as those of the preceding segments. The shell of the egg is produced from secretions of its **tubular glands**. The **mucosa** is thrown into longitudinal, **leaf-shaped folds**, which are covered by a ciliated pseudostratified, columnar epithelium. Ducts of the glands can sometimes be seen to pierce the epithelium. The **muscularis** is well developed, especially the longitudinal layer.

The **vagina, slide C-27**, is a short, narrow duct. Its **muscularis** is well developed, especially the circular layer. Its **mucosa** is thrown into numerous tall, narrow folds bearing many small secondary folds. The epithelium is ciliated pseudostratified columnar with mucous cells. A **serosa** is present. **Sperm-host glands** are present in the mucosa of the vagina and **may be visible** on your slide. They are located in the connective tissue of the mucosa of the vagina near the junction of the latter with the uterus. After insemination, sperm appear in compact masses within the glands. The vagina opens into the urodeum of the cloaca.

Examine **slide 88** of a **resting (anestrus) uterus**. Identify the **endometrium**, **myometrium** and **serosa** (the serosa is also known as the **perimetrium**). Note the cuboidal epithelium, uterine glands, and the very cellular lamina propria. Examine **slide 89 of a uterus in estrus**. Note the highly developed **uterine glands** (coiled tubular), the thickened muscle layer, and increased size of the blood vessels. The epithelium of the uterus during proestrus and estrus is columnar.

Examine the **inactive mammary gland of the cow, slide 91**. With the l0X objective, observe that the lobules consist of clusters of **intralobular ducts** surrounded by a **richly cellular connective tissue**. With the 40x objective, examine the epithelial lining of these ducts. Is it simple or stratified? Are there any fat cells present in the interlobular connective tissue? Are there any interlobular ducts? Are there any lymphatic vessels?

Examine **slide 90, an active mammary gland from the cow**. Note the interlobular connective tissue, the interlobular ducts, the secretory acini and the intralobular connective tissue. Note the presence of secretion in the acini. Sloughed cells with dark nuclei form part of the secretory product. Cells of acini of an active gland are generally vacuolated and “moth-eaten” in appearance because their cytoplasmic lipids were extracted during processing. Some of the flat nuclei seen surrounding the acini belong to myoepithelial cells. Rounded concretions of casein and cellular debris will be seen in some acini. Such masses are called **corpora amylacea**.

Examine **<u>slide 92, an inactive mammary gland from the cat</u>**. Note the similarity between this slide and slide 91. Slide 92 includes the overlying skin.

Examine the **<u>teat, slide 93</u>**. Some of these are sections through the **teat canal** are lined by stratified squamous epithelium. Other slides are through the **teat sinus**, which is lined by a bistratified columnar epithelium.

LABS 16 & 17 URINARY SYSTEM

 # 71 – kidney
 # 73 – kidney
 # 74 – kidney
 # C-18 - kidney (avian)
 # 75 - ureter (horse)
 # 76 - urinary bladder

Slides 71, 73 and 74 (mammalian kidneys) are stained with H&E and silver, respectively. Locate **cortex** and **medulla** and the prominent **pyramid** and **papilla**. The latter two structures may be missing on slide 71. Both the cortex and medulla are formed mainly of numerous **uriniferous tubules**. Between the tubules is an extensive capillary network. In the cortex, groups of radially arranged tubules form the **pars radiata (cortical ray or medullary rays),** consisting of **collecting tubules** and straight portions of **nephrons**. The **pars convoluta (cortical labyrinth)** are located between the rays and consist of **renal corpuscles** and numerous **proximal and distal convoluted tubules**. Proximal convoluted tubules are longer than distal tubules and comprise the major portion of the cortex. The apical ends of their epithelial cells present a scalloped appearance and possess a **brush border**. Epithelial cells of distal convoluted tubules lack a brush border and have a flat, smooth apical surface. These characteristics are best seen on slides 71 and 73. Can you locate a **macula densa**? In the medulla, you should be able to identify **ascending and descending thick segments of the loops of Henle** as well as **thin segments of the loops of Henle**. **Collecting tubules** should also be identified. Their cuboidal cells have distinct boundaries and clear, faintly stained cytoplasm. Numerous **capillaries** will also be in evidence.

Slide C-18 of the **avian kidney** has a **very different organization** so far as the lobes, lobules and blood vessels are concerned and also so far as the spatial relationship of the cortex and medulla is concerned. In the bird kidney, the cortex and medulla do not form a continuous outer and inner strata as in the mammalian kidney. Instead, **large areas of cortex enclose small cone-shaped islands of medulla**. The bird kidney contains **two types of nephrons**. **Cortical nephrons** (reptilian type), which lack loops of Henle and possess small renal corpuscles, are found in the cortex. **Medullary nephrons** (mammalian type) are less common. These have larger renal corpuscles and loops of Henle which extend into the medullary cones.

In the cortex you should be able to find **proximal and distal convoluted tubules** as well as **macula densae**. These structures look like the mammalian equivalents. **Collecting ducts** are evident in the medulla.

Slide 75 is a section of **horse ureter**. Your slide may have two sections. One of the sections is from the proximal third while the other is from the caudal third. Note: 1. **many folds**, 2. **transitional epithelium**, and 3. **muscularis**. Note that the muscularis has inner longitudinal, middle circular and a few bundles of outer oblique or longitudinal fibers.

Both sections have essentially the same structure but the equine renal pelvis and proximal third of the ureter have simple **branched tubuloacinar mucous glands in the lamina propria**. Note their presence in one of these sections.

Examine the following three layers of the <u>**urinary bladder**</u>, <u>**slide 76**</u>: 1**. mucosal epithelium** (what type is it?); 2. **lamina propria**; and 3. **muscularis,** composed of helically wound bundles of smooth muscle.

LAB 18 THE EYE

 # 56 – dog eye
 # 100 - cat eye

Slides 56 and 100 are **sagittal sections of an eye** (dog or cat) and include all structures present in the **bulbus oculi** (eyeball). The globe of the eye has three concentric layers: i) an outer **fibrous layer** that includes the **sclera** and **cornea**; ii) a middle **vascular layer** - the **uvea** - that includes the **choroid**, **ciliary body and the iris**; iii) and an inner **nervous layer** that includes the **photoreceptive retina** and the **non-photosensitive epithelium** that covers the ciliary body and posterior iris**.**

Recall that the eye has three chambers: the **anterior**, **posterior**, and **vitreous**. Review where these chambers are on your slide, the anatomical structures that define them, and their relationship to the **iris** and **pupil**.

Beginning in the caudal eye, find the **optic papilla** (also known as the **optic disc** or **blind spot**) and observe, starting from the outside, the following layers: **sclera**, **choroid**, and **retina**. The **optic nerve pierces the sclera** at the **lamina cribrosa sclerae**, the perforated region of the sclera through which the nerve passes. The **choroid** is the middle, highly pigmented, vascular layer. The numerous granules of black-brown pigment observed here are located in the bodies and processes of the many **melanocytes**. Note the vascular outer part of the choroid. These vessels provide nutrition to the outer layers of the retina. Next, look for the reflective layer of the choroid, the **tapetum lucidum**. It is located just outside the retina, mostly dorsal to the optic nerve. In some slides, the tapetum has conveniently separated from the retina (an artifact from the tissue preparation), creating an artificial gap. Note that the pigment epithelium of the retina is sparse or lacking where the tapetum lucidum is located. Follow the extent of the tapetum lucidum dorsally and anteriorly until it disappears. The tapetum lucidum is present in most mammals but is lacking in humans and pigs.

The **retina** consists of two regions: the posterior retina, which is photoreceptive (or "sensory"), and the anterior retina, which is nonphotoreceptive (or "nonsensory"). The posterior (photoreceptive) retina is many cell layers thick (outlined below), but the anterior retina (non-photoreceptive) is a simple bi-layer of epithelial cells. The junction between the sensory and nonsensory retina is known as **ora ciliaris retinae** (or the "ora serrata" in humans). Find the ora ciliaris retinae on your slide.

Observe the **photosensitive retina**. With the aid of the figures in your text, handout, and website, identify the following ten tissue layers of the retina and understand their functional relationships:

1. **pigment epithelium** (adjacent to the choroid)
2. **layer of rods and cones**
3. **outer limiting membrane** (not always distinct)
4. **outer nuclear layer**
5. **outer plexiform layer**
6. **inner nuclear layer**
7. **inner plexiform layer**
8. **ganglionic cell layer**
9. **optic nerve fiber layer**
10. **inner limiting membrane**

Observe the **nonphotoreceptive retina**. This is an epithelial bi-layer that lines the caudal sides of both the ciliary body and the iris. The portion of the retinal bi-layer lining the back of the ciliary body is called the **pars ciliaris**

retinae; the portion of the retina extending over the caudal surface of the iris is the **pars iridica retinae**. Find these regions on your slide.

On the posterior surface of the ciliary body, the epithelial bi-layer that makes up the nonphotoreceptive retina consists of the following:

Layer 1: the **ciliary epithelium,** a superficial layer of **columnar epithelial cells**. This is a continuation of the neural retina.
Layer 2: a basal layer of **simple cuboidal epithelium with melanin granules**. This is a continuation of the pigment epithelium of the retina.

Layer 1, the ciliary epithelium, is called the superficial layer because it is closest to the lumen of the vitreous chamber. Layer 2 is closest to the tissue of the ciliary body, and is considered the basal layer. Distinguish these layers on your slide – look just in front of the ora ciliaris retinae. Note the pigmentation in Layer 2, which is basal here, will become more superficial in the iris.

Locate the **iris**. The iris is the most anterior part of the **uveal tract**. It forms a thin, contractile diaphragm with a central aperture, the **pupil**. The base of the iris is attached to the anterior portion of the ciliary body. The connective tissue stroma of the iris contains many **melanocytes** and blood vessels. The **stroma** contains circumferentially arranged bundles of smooth muscle that form the **sphincter (constrictor) muscle**. The anterior surface of the iris is not covered by an epithelium, but rather by a discontinuous layer of stromal cells (fibroblasts and melanocytes). The posterior surface is covered by a bilayer of epithelial cells, the pars iridica retinae, which represents the most anterior continuation of the nonphotosensitive portion of the retina. It consists of:

1. a superficial layer of **pigmented columnar cells**
2. a basal layer of partially pigmented **myoepithelial cells**

The myoepithelial cells are elongated, radially arranged, contractile cells that form the **dilator muscle** of the iris. The myoepithelial cells have an apical, pigmented portion containing the nucleus and a basal, nonpigmented portion. The nonpigmented regions of these cells border the stroma and appear as an acidophilic band.

Locate the cornea and study the characteristics of its layers as follows:

1. anterior epithelium
2. Bowman's membrane (not distinct in domestic mammals)
3. substantia propria (stroma)
4. Descemet's membrane
5. posterior mesenchymal epithelium (single squamous cell layer)

Why is the cornea completely transparent in the living eye? Is it vascularized?

Locate the angle where the cornea, ciliary body, sclera and iris meet, i.e., the **filtration angle**. A meshwork at the filtration angle encloses the **spaces of Fontana**. Fluid drains through these spaces and eventually reaches the scleral venous plexus, which you may be able to see in your section. Refer to your text for clarification and an understanding of the functional significance of these structures.

Locate the **lens**, the substance of which is sure to be fragmented to some extent by the sectioning process. Most sections will reveal at some part the following: 1. **lens capsule**, 2. **anterior epithelium** (is there a posterior epithelium?), 3. **lens substance** consisting of elongated cells (lens fibers). Look at the equator or marginal zone and see if you can follow the transition from epithelial cells to lens fibers (recall that the equator of the eye is vertical, not horizontal, so look for this transition at the most dorsal or ventral aspect of the lens). Is the lens vascularized?

Find the margin of the cornea and note the change in its epithelium where it is continuous with the **epithelium of the conjunctiva** (the conjunctival epithelium may not be present on all slides). On some slides, the epithelium of the conjunctiva shows numerous **goblet cells**.

LAB 19 ORAL CAVITY, PHARYNX AND ESOPHAGUS

 # 36 - lip
 # 37 - tongue (cat)
 # 38 - tongue (rabbit or cat)
 # C-2 - tongue (avian)
 # 40 - pharynx (dog)
 # C-3 - pharynx (avian)
 # C-4 - esophagus (avian)
 # 41 - esophagus (dog)
 # C-5 - crop (avian)

Slide 36 is a section of the **lower lip of a dog**. Study it first with your 4x objective noting the **cutaneous region** (hairs present) and the **oral mucosa**. The central portion of the section is composed of **skeletal muscle (orbicularis oris)**. With higher magnification, study the variation in the epithelium and in the connective tissue **papillae**, which are more conspicuous in the oral mucosa. **Labial salivary glands** are visible on a few slides in the class collection. **Review the structure of hair follicles, sinus hairs, sebaceous and sweat glands,** which can be observed in the cutaneous region.

Slide 37 is a sagittal section of the **tip of a cat's tongue**, which shows numerous **filiform papillae** with **keratinized spines** and an occasional **fungiform (mushroom-like) papillae** on the dorsal surface. The ventral surface is covered by a **nonkeratinized, stratified squamous epithelium**. Observe the arrangements of the **skeletal muscle cells** of the lingual musculature.

Depending on your slide box, **slide 38** is either from a **cat or rabbit's tongue**. **Circumvallate papillae (cat)** or **foliate papillae (rabbit)** should be found. **Salivary glands –** serous, mucous, or mixed – should also be observed. Locate **taste buds** embedded in the epithelium of the papillae. These ovoid structures open into the oral cavity through taste pores, which are sometimes visible. Three types of cells (sensory, supporting and stem cells) are present in each taste bud. However, their identification is not feasible in routine histologic preparations. Sensory nerve endings (also not visible) terminate on the sensory cells.

On the **avian tongue**, **slide C-2**, there is a complete outer layer of stratified squamous epithelium which is thicker and nonkeratinized on most of the dorsal surface and thinner and keratinized on the ventral surface. The tip of the dorsal surface is also keratinized. Papillae are not evident in this section. They are present in the more posterior region of the tongue as is most of the musculature. Unlike mammals, there is a bone in the bird's tongue, the bone being an extension from the **hyoid apparatus**. This section just shows the more anterior cartilaginous region. This rigid support is very important in birds with long protrusible tongues, such as woodpeckers, enabling them to reach insects in small crevices.

<u>Slide 40</u> was taken from the posterior dorsal wall of the **<u>dog's pharynx</u>** near the entrance to the esophagus. Identify the type of **epithelium** present, and note the character and arrangement of the **lamina propria, submucosa, muscular layer, and fibrous adventitia**. **Lobules of mucous and mixed glands** may be observed in the muscular layer.

<u>Slide C-3</u> is of the **<u>avian oropharynx</u>**. It is lined by thick, **nonkeratinized, stratified squamous epithelium**. **Salivary glands** are located in the base of the **lamina propria** or within the **submucosa**. Skeletal muscle of the **hyoid apparatus** may be present on this section indicating that it was taken from the ventral portion of the pharynx.

Examples of **avian salivary glands** can be found on **<u>slides C-3 (pharynx) and C-4 (esophagus)</u>**. They are found throughout the oropharynx, the posterior tongue and in the esophagus. They are aggregates of many compound tubular glands of the mucous type. They usually empty into a common cavity with a common duct to the outside. Some single glands may empty their secretions by a separate duct. The common ducts have a low columnar epithelium. There are no serous or mixed glands in the chicken. Although taste buds occur in birds in the vicinity of the common ducts of the salivary glands, they are not present our preparations.

<u>Slide 41</u> is a section of a **<u>dog's esophagus</u>**. Observe the **stratified squamous epithelium**. The degree of keratinization varies depending on the diet of the animal. It is more pronounced in animals that feed on hard, dry food, e.g., herbivores. With low magnification, observe the branched **tubuloacinar mucous** (the predominant type) or **mixed glands** that, in the dog, form a continuous stratum in the **submucosa**. Other species have these glands but they do not always extend the entire length of the esophagus. The **muscularis mucosae** is made up of longitudinal bundles of smooth muscle, which, in the dog, becomes a continuous sheet of muscle in the vicinity of the stomach.

The **muscularis externa** consists of two layers of skeletal muscle, **inner circular** and **outer longitudinal**, with some smooth muscle near the caudal end. The inner circular layer becomes thicker as it progresses towards the stomach. In some species, e.g., the horse, pig, and cat, the entire caudal 1/3 to ½ of the muscularis externa is composed of smooth muscle. In the dog and cow, the muscularis externa remains predominantly striated the entire length of the esophagus.

The **<u>avian crop</u>**, **<u>slide C-5</u>**, is a distensible part of the lower esophagus (slide C-4). The crop functions as a storage area, primarily in herbivorous species. It enables birds to eat and store a large amount of food quickly and then digest it later in a more sheltered environment.

The tissue layers are similar to the esophagus in other species. The thickness of the component layers is dependent on the diet. **Mucous salivary glands** are present throughout the esophagus. They are more numerous in the upper portion and serve to lubricate the food as it passes through. There are **lymphatic nodules** frequently found in association with these glands. The crop is almost identical in structure to the rest of the esophagus. Glands are present in the region where the crop joins the esophagus but are absent from the rest of the crop. During the reproductive season, the crop epithelium in the Columbiformes (pigeons and doves) rapidly proliferates and desquamates fat-laden cells. This epithelial product, known as crop- or pigeon-milk, is fed along with food particles to the nestlings. Greater flamingos and emperor penguins also produce a nutritive fluid and are the few examples of birds producing a substance similar to mammalian milk to feed their young.

LAB 20 STOMACH

 # 48 - rumen
 # 49 - reticulum
 # 50 - omasum
 # 42 - esophageal stomach
 # 43 - esophagus / cardiac stomach

 # 44 - fundic stomach
 # 45 - fundic stomach
 # 47 - stomach (comparative)
 # C-6 - proventriculus (avian)
 # C-7 - ventriculus (avian)

Histologically, **four distinct regions of the stomach** can be identified: **esophageal, cardiac, fundic, and pyloric**. The **esophageal (nonglandular) region** is lined by **stratified squamous epithelium**. The **cardiac region** is characterized by **cardiac glands**, which are branched, tubular and coiled. The secretory cells are columnar, mucus-secreting cells. The **fundic region** is also **glandular**, but the fundic glands are longer than cardiac glands and they are less frequently branched. The gland cells of the fundic region **secrete gastric enzymes and HCl**. The specific cell types will be reviewed in lecture. In the **pyloric region**, the **glands** are long and the cells are primarily of the mucous variety. Different species have different proportions of these regions – see the diagram below (from Banks, 1981).

The ruminant stomach has four chambers: **rumen, reticulum, omasum, and abomasum**. The **forestomach** consists of the first three chambers. It is lined by stratified squamous epithelium and is **nonglandular**. The **fourth chamber** is **glandular** and resembles the glandular stomach of other animals. Both forestomach and glandular stomach are derived from the stomach primordium of the embryo.

<u>Slide 48</u> is a section of **<u>rumen</u>** from a cow. Study the section grossly first and note the presence of short and long **papillae**. Now study the slide with the l0X objective and note the following: presence of **keratinized epithelium** and **intercellular bridges**. The latter resemble those of the epidermis. Note that **glands and a muscularis mucosae are lacking** and that there is no distinct line of separation between the **lamina propria** and **submucosa**. However, the connective tissue fibers of the lamina propria are finer. Adjacent to the connective tissue of the submucosa is the thick **muscularis externa** consisting of smooth muscle. A **serosa** can be seen external to the muscular externa.

In the **<u>reticulum</u>**, **<u>slide 49</u>**, the mucosa is thrown into long and short **folds not papillae**, as in the rumen. The short folds have cores of **lamina propria** only, while the tall folds have cores of **lamina propria and submucosa**. The tall folds also have **bundles of smooth muscle** near their free end. The tall folds form the honeycomb part of the reticulum. Numerous **papillae** project laterally from the walls of the tall folds. The **epithelium is keratinized**.

<u>Slide 50</u> is a section of the **<u>omasum</u>** of a cow. Look at the section grossly and observe the **laminae omasi (folds).** The **epithelium is keratinized**. The **lamina propria, submucosa, muscularis mucosae and muscularis externa** extend up into the longer, primary laminae. Within the primary laminae note that the smooth muscle core is arranged in three layers, the central core of which is derived from the muscularis externa. **Secondary papillae** may be visible on the surface of the laminae.

<u>Slide 42</u> is a section of the **<u>esophageal (nonglandular) region</u>** of the horse stomach. Its **epithelium is keratinized,** a **muscularis mucosae** is present, and **glands are absent**. Identity the various layers and study their characteristics.

<u>Slide 43</u> is a section of both the **<u>esophageal and cardiac regions</u>** of the pig stomach. The **thick, stratified squamous epithelium (keratinized) and absence of glands** characterize the esophageal region. Moving across the slide, note the abrupt change to a **simple columnar epithelium** upon entering the cardiac region. **Gastric pits (foveolae),** depressions of the mucosal epithelium, are present. **Simple, tubular mucous glands** open into the epithelium at the base of the pits. **Lymphatic nodules** may be evident in your section. Identify the various layers of the stomach wall.

<u>Slides 44 and 45</u> are both from the **<u>fundus of a dog's stomach</u>**. First, study slide 44 and note the layers of the stomach. The **mucosal epithelium** is composed of a layer of simple columnar cells that secrete mucus. The shape of these cells and the chemical composition of the mucus is not the same as the goblet cells found in the intestines. Examine the **gastric glands**, which open into the **gastric pits**. These are simple tubular glands that can be divided into four regions: **fundus (base), body, neck, and isthmus**. The isthmus is the open end of the gland. It is continuous with the neck, which is more constricted. The body of the gland is the main tubular section and terminates at the base.

Study the cells comprising the glands. **Chief cells** secrete pepsinogen and are basophilic. Their pyramidal shape and basally located nucleus distinguish them from **parietal cells**, which are large, round, acidophilic cells that secrete HCl. **Mucous-neck cells** line the neck of the gland. They are cuboidal to low columnar and secrete a mucus that differs from that of the mucosal epithelium. The enteroendocrine cells (argentaffin cells) occur wedged between the basement membrane and chief cells but can be seen only following the use of special stains. Argentaffin cells produce serotonin, histamine, glucagon, epinephrine and gastrin. Note that delicate strands of smooth muscle of the muscularis mucosae extend upward between the glands. Fibers within the muscularis mucosae may either run in a circular or longitudinal fashion. Also note the thick muscularis externa.

Slide 45 is a section of the fundus that was stained by the periodic acid-Schiff (PAS) method. Mucigen, mucus not yet secreted, stains a brilliant red following application of this method, enabling you to locate **mucous-neck cells** as well as the mucus-secreting cells of the mucosal epithelium. NOTE: MUCUS = THE NOUN, MUCOUS = THE ADJECTIVE.

Slide 47 has three sections from the **cat stomach**. The top is from the **pyloric region**, the middle is from the **dark zone of the fundic region**, and the bottom is from the **light zone of the fundic region**. Compare the fundic region with that of the dog 44. They are similar except for the presence of a **lamina subglandularis** in the cat. The lamina, located between the base of the **glands** and the **muscularis mucosae** is actually composed of two layers. The inner one, the stratum granulosum, consists of fibroblasts. The outer layer, the stratum compactum, is composed of densely woven collagenous and elastic fibers. It appears as a pink band immediately adjacent to the muscularis mucosae. Note that the **muscularis mucosae is arranged into three layers: inner circular, middle longitudinal and outer circular** in the fundic and pyloric regions of the stomach. The lamina is also found in the small intestine. What might the advantage of this layer be to a cat? In the dog, the lamina is less pronounced or nonexistent. **Slide 46** is a section of the **pyloric region of the cat's stomach**. Note the type of glands present and the depth of the **gastric pits**.

The avian stomach is divided into a glandular portion, or **proventriculus**, and a muscular gizzard, or **ventriculus**. Scan your slide **C-6** of the **proventriculus** from the chicken with the 4x objective. Note the **lobules** of compound tubular glands which are located in the **submucosa**. The lobule consists of plates of cuboidal epithelial cells (**oxynticopeptic cells**) arranged on thin layers of connective tissue that radiate around a central lumen. Unlike other epithelial cells, these cells are not held together by apical junctional complexes. The resulting gaps between the cells give the epithelium a serrated appearance. They secrete both HCl and pepsinogen and therefore combine the functions of both the chief and parietal cells in mammals. Enzymatic secretions collect in a central cavity lined by columnar epithelium and from there are discharged through a duct into the lumen of the proventriculus. Grossly, the orifice of the duct is surrounded by concentric rings of mucosal folds called **plicae**. Depressions between these folds are known as **sulci**. On your slide, find a gland with a duct that communicates with the luminal surface. In longitudinal section, the folds of mucosa on either side of the duct have the appearance of villi. The mucosal epithelium is columnar at the apex of the folds but decreases in height to appear cuboidal at the bottom of the sulci. **Lymphocytes** infiltrate the **lamina propria**, especially around the duct. The **muscularis mucosa is not a continuous layer**. It is present as small bundles of smooth muscle that may be difficult to distinguish from the surrounding connective tissue.

The **gizzard (ventriculus), slide C-7**, can have a thick muscular wall as in granivores (grain eaters), or can be a thin-walled storage sac as in carnivores, piscivores (fish eaters) and frugivores (fruit eaters). In your section of the gizzard, observe the thick region of smooth muscle, which is very important in grinding food. On the mucosal surface there is a bright pink layer of filamentous, keratinoid protein. This **tough lining** is well developed in birds that eat rough foods. It is secreted by branched, tubular glands that lie along, and at the base of, the gastric pits. These pits project downward from the mucosa. The secretion is hardened by HCl and is anchored to the gizzard wall.

LAB 21 SMALL AND LARGE INTESTINE

 # 51 - stomach/duodenum
 # 52 - jejunum
 # 53 - ileum
 # C-8 - duodenum (avian)
 # C-9 - ileum (avian)
 # 55 - colon
 # 57 - rectum
 # 58 - recto/anal junction
 # C-37 - ceca (avian)
 # C-10 - ceca (avian)
 # C-11 - large intestine (avian)

While the function of the stomach may be to initiate the digestive process, it is really the small intestine that does the bulk of the digesting and absorbing of nutrients. It is well adapted to meet these demands. The surface area of the intestine is many times compounded by the presence of **plicae, villi,** and **microvilli**. Furthermore, it is a very long tube. All of these factors contribute to its efficiency. **Plicae** are permanent folds visible upon gross inspection of the lumen of the small intestine. **Villi**, visible with the microscope, are fingerlike projections of the mucosa. **Microvilli** are fingerlike projections of the plasma membrane of intestinal epithelial cells. Collectively, microvilli form the **striated border**.

Note the **simple tubular glands (crypts of Lieberkühn**) which empty at the base of the intestinal villi. Observe the **columnar absorptive and goblet cells** of the epithelium. Paneth cells are found at the base of the crypts in ruminants, horses and humans but not in other species. See the demonstration slide of Paneth cells. Read about these cells in your text. Enteroendocrine (argentaffin) cells are present in the intestine but are demonstrable only with special staining - see the slide on demonstration.

The small intestine consists of three regions: **duodenum**, **jejunum** and **ileum**. Your slide box contains an example of each.

<u>**Slide 51**</u> is a section through the <u>**pyloric end of the stomach and the duodenum at the transition**</u> point. Note that the surface epithelium of the stomach does not have a striated border or goblet cells, as does the duodenum. Can you find the approximate point of transition between stomach and duodenum on your slide?

Observe the **muscularis mucosae** and **submucosa**. Looking at the duodenum you should be able to locate the **duodenal or Brunner's glands** in the **submucosa.** Some of the glandular elements pass from the submucosa to the mucosa thereby interrupting the muscularis mucosae. Also, some of the glands extend into the pyloric stomach. What type of secretion do these glands produce? Projections of the mucosa extend outward as **villi**. In the **lamina propria** you should see blood vessels and smooth muscle fibers within a bed of loose connective tissue. Note the thick **circular layer** of the **muscularis externa** and the thinner **longitudinal layer**.

Examine **<u>slide 52</u>** of the **<u>jejunum</u>** noting the **slender villi** and the absence of Brunner's glands. At this time review the cell types of the **lamina propria**. **Plasma cells** are particularly abundant and easily identifiable. **Lymphocytes** and eosinophils should also be easy to find. Identify the **crypts**, the scanty **muscularis mucosae**, the **submucosa**, and the **muscularis externa**. Again note the difference in thickness between the internal and external layers of the muscularis externa.

In the **<u>ileum</u>**, **<u>slide 53</u>**, there is a decrease in the number of absorptive cells and an increase in the number of **goblet cells**. Note also the aggregations of many **lymph nodules** into a **Peyer's patch**. The patches usually are located on the antimesenteric side of the ileum. You should also be able to see a well defined **muscularis externa**, and myenteric and submucosal nerve plexi. The **myenteric (Auerbach's) plexus** is located between the outer and inner muscle layers of the muscularis externa. The **submucosal (Meissner's) plexus** is in the submucosa.

Histologically, the **avian small intestine** cannot be neatly subdivided as it is in mammals. Grossly, the duodenum exists as a short U-shaped loop that begins at the pylorus. The pancreas is contained within this loop. After this is an elongate jejunum, as in mammals. The remainder of the small intestine, running parallel to the length of the ceca, is the ileum. In the **<u>duodenum</u>**, **<u>slide C-8</u>,** the **mucosa** is organized into numerous long **villi** with **crypts of Lieberkühn** at their bases. Tall **columnar epithelial cells** are the major cell type covering the surface of the villi. At the base, numerous **goblet cells** can usually be found. In the **<u>ileum</u>**, **<u>slide C-9</u>,** the only histologic change is a gradual shortening of the **villi** and sometimes a decrease in the number of **crypts.**

Observe the **<u>colon</u> (large intestine), <u>slide 55</u>**. The main functions of this segment of the digestive tract are water absorption, formation of feces, and lubrication of the mucosal surface. There are **few folds** and **no villi** but there are **deep crypts of Lieberkühn**. These are lined by **absorptive cells, goblet cells**, and a small number of enteroendocrine cells. The **lamina propria** is rich in l**ymphocytes** and **lymphatic nodules**. The latter may be seen extending into the **submucosa**.

<u>Slides 57 and 58</u> are sections of both the **<u>rectum</u>** and **<u>its junction with the anus</u>** of a dog. Find the terminal end of the rectum with short **crypts** and **numerous goblet cells**. At the junction of the rectum with the anal canal, the lining **epithelium abruptly changes from simple columnar to stratified squamous** and the **crypts disappear**. On some slides the epithelium of the rectum may be missing. In the **submucosa** at the rectal-anal juncture are the **anal glands** which are coiled tubular glands (modified sweat glands). The skin seen on these sections contains **compound hair follicles, sweat glands, and sebaceous glands**. This is the skin peripheral to the anus. In the deeper zone of the **dermis** note the presence of **hepatoid cells**. These resemble hepatocytes, hence their name. Clusters of them in this location are referred to as **circumanal glands**. They may represent abortive sebaceous glands. In the center of the section you will see a cavity lined by keratinized, stratified squamous epithelium. This is the lining of the **anal sac**.

Anal sacs are bilateral structures. Each empties into the terminal end of the anal canal by a duct, which may not be visible in your section. They are found in many carnivores and contain a foul-smelling product. They often become inflamed and even abscessed. In the skunk, they are the scent glands. Tubular, apocrine glands (**glands of the anal sac**) will be seen surrounding the anal sac. Observe that the **internal circular layer** of the muscularis externa of the rectum thickens to become the **internal anal sphincter** near the rectal-anal juncture. The **longitudinal layer** gradually disappears. Peripheral to the anal sac, note the mass of skeletal muscle, the **external anal sphincter**.

In the bird, the **ceca** are paired, blind pouches that project anteriorly from the junction of the ileum and colon. Their purpose and function have not been well described. There are a number of different histologic types among species: 1. the intestinal type, with a similar structure to the adjacent ileum plus lymphoid infiltration; 2. a secretory glandular type which occurs in owls and has numerous goblet cells; and 3. a cecal tonsil in which lymphoid tissue dominates the architecture. **Slide C-37** is an example of this third type. **Slide C-10** is an example of the intestinal type. In many species, only vestiges of the ceca remain.

The **large intestine**, **slide C-11**, extends from the ileocecal junction to the cloaca. It does not differ markedly from the small intestine except in the size of the villi and crypts. Its main functions are fecal storage and water absorption.

LAB 22 LIVER, GALLBLADDER AND PANCREAS

 # 64 - liver (pig)
 # 65 - gallbladder (pig)
 # 66 - liver (cat)
 # 62 - pancreas

The **liver** has a multitude of functions, some of which include: bile production (important in fat digestion); lipid, protein, and carbohydrate metabolism; lipid, glycogen and vitamin storage; iron metabolism; synthesis of blood proteins; and biotransformation of toxins, drugs, and hormones.

The cells that accomplish the above activities are called **hepatocytes**. They are the basic structural components of the liver. Hepatocytes are grouped into **cords (plates)**, which are separated from each other by **sinusoids**. The cords and their associated sinusoids radiate from a **central vein**. One central vein, the cords surrounding it, and the sinusoids that drain into the vein, constitute a **classic hepatic lobule**. The liver is made up of thousands of such lobules.

Begin your study of the liver with **slide 64**, the **pig liver**. The hepatic lobules are particularly distinct in pig liver because of the presence of **interlobular connective tissue**. Scan the slide for a good example of a lobule with its **central vein**. Then, with higher magnification, examine the general pattern formed by the **hepatocytes** and the **sinusoids**. Hepatocytes have acidophilic cytoplasm and a euchromatic nucleus. Occasionally a **binucleate cell** may be seen. Distinct cell margins are usually discernible. The nuclei of the cells of the sinusoidal endothelium can be identified by their slender form. Kupffer cells (macrophages) are present within the sinusoids where they occur on the surface of the endothelium. These cells are best seen after they have ingested particulate material. See the demonstration specimen.

Find an area where the corners of three lobules meet. This is a **portal tract (area).** Each tract contains one or more branches of a **portal vein, hepatic artery, bile ductule, and lymphatic vessel**. These components are supported by a connective tissue framework. Consult your text and lecture notes for a review of the blood circulation through the liver. Be sure you understand the direction of flow of the bile and the blood within the lobule. The figure shown on the following page may help you (from Bloom & Fawcett, 1968).

Bile travels from the **hepatocytes** to the **bile duct** via canaliculi. These structures usually require special staining to be seen. A demonstration slide will be on display for you to examine. What type of epithelium lines the **bile ductules** in the portal tracts? Scan your slide for larger bile ducts. What type of epithelium do they have?

Now look for comparable structures in the liver of the **cat, slide 66**. **Note the lack of distinct lobulation**. Despite this, you should still be able to pick out a **lobule** with its **central vein** and adjacent **portal tract**.

The **gallbladder** stores and concentrates bile. **Slide 65** has a piece of **gallbladder**. Study the layers of this organ: **epithelium, lamina propria, muscularis, adventitia**. The epithelium has a **striated border**. How might this be important to the function of the gallbladder?

The **pancreas, slide 62**, has both endocrine and exocrine functions. In part, it is a **compound tubuloacinar, exocrine gland** with parenchymal cells of the **serous** type. This part is organized into **lobules. Acini, centroacinar cells, and intercalated ducts** should be found. The latter are very long and are the only ducts found within the lobules. They are lined by cuboidal or squamous epithelium. The intercalated ducts join **interlobular ducts**, which are lined by a simple columnar epithelium. Using the 40x objective, study the detail of the acinar cells noting particularly the difference between the apical, zymogenic portions of the cells and their basophilic basal regions.

Small clusters of pale-staining cells, the **islets of Langerhans**, constitute the **endocrine portion** of the pancreas. The islets will be found scattered randomly amongst the acini.

LAB 23 RESPIRATORY SYSTEM

 # 69 - trachea
 # C-15 - trachea (avian)
 # 16 - lung
 # 70 - bronchus and lung
 # 72 - lung
 # C-17 - lung (avian)

1. Begin by studying the **mammalian trachea (rabbit)**, **slide 69**. Identify the **pseudostratified ciliated columnar epithelium, lamina propria, submucosa (no glands present in the rabbit), hyaline cartilage, adventitia (consisting of loose connective tissue) and the trachealis muscle (smooth)**. The trachealis muscle extends between the ends of the **c-shaped tracheal cartilage** and terminates on the **perichondrium.** Now compare the mammalian trachea to the **avian trachea**, **slide C-15**. Note that in the avian slide, there are **intra-epithelial, multicellular mucous glands of the simple saccular (alveolar) variety**. If the section on your slide has one thick and one thin ring of cartilage, this is due to the telescoping of one within another. The **cartilage rings are complete in the bird** while in mammals they are C-shaped, as noted previously. In some birds, e.g., geese and ducks, the cartilage becomes ossified.

2. **Slides 16 and 70** are of the **mammalian lung**. Look at slide 70 with the naked eye and locate the **bronchi**. Examine these with the 4x and 10x objectives and note the characteristics of the **epithelium, lamina propria, muscularis, submucosa, glands** and the **fibroelastic membrane** containing the **cartilage plates**. Now look for sections of some **smaller bronchi** and notice structural changes which occur as the bronchi get smaller. Notice particularly the **longitudinal folds** present in the smaller bronchi, the **disappearance of the glands**, and the **decrease in the amount of cartilage**. Use higher magnification to study structural detail.

Now scan other portions of slide 70 (also examine slide 16) and observe the structure of **bronchioles**. From largest to smallest, respectively, the **bronchioles are named: primary, secondary, tertiary (terminal) and respiratory. All lack cartilage.** Primary and secondary bronchioles are lined by a **ciliated columnar epithelium**; while terminal and respiratory bronchioles are lined by a **cuboidal epithelium**, which rarely has cilia. **Smooth muscle** forms a part of the bronchiole wall but decreases in amount as the bronchioles decrease in size. There are several layers of smooth muscle in primary bronchioles, fewer in secondary bronchioles and only a single layer in terminal bronchioles. Respiratory bronchioles (best on slide 16) have only an occasional smooth muscle cell. Note that the mucous membrane of primary and secondary bronchioles is folded, but beyond the secondary bronchioles folding does not usually occur. **Respiratory bronchioles possess alveoli,** here and there, in their walls. **Alveolar ducts**, whose walls are made up of alveoli, connect respiratory bronchioles with **alveolar sacs.** How are alveolar sacs distinguished from alveolar ducts?

3. **Slide 72**, **the mammalian lung**, is stained with resorcin-fuchsin so that all **elastic fibers** are stained blue-black. Note the distribution of elastic fibers around the bronchi, bronchioles and alveoli.

4. Avian lungs are very different from mammalian lungs. The avian respiratory tract consists of the following: **1. the ducts (the trachea, and the primary and secondary bronchi), 2. the region for gas exchange (parabronchi, atria, and air capillaries), and** 3. the pump which moves the air through the system (air sacs).

The air sacs are membranous, poorly vascularized continuations of the lung. There are 4 paired and 1 unpaired groups of air sacs within the avian thoracoabdominal cavity, with part of their structure extending as diverticula into certain bones, e.g., humerus, pelvis, and vertebral column. As birds do not have a diaphragm, the air sacs are responsible for the movement of air through the lung. The air sacs do not, however, participate in gas exchange themselves. The system works like a bellows, with all the air sacs expanding or contracting together during ventilation. When the bird inhales, the air sacs expand, drawing air into and even through the lungs. When the bird exhales, the air sacs collapse, forcing air out of the lungs and out the trachea.

Unlike mammals, where inhaled air dead ends in the alveoli of the lung, inhaled air in the avian respiratory system is routed through a complex and coordinated branching system of bronchi and air sacs before it reaches the lung. Gas exchange in the avian lung takes place in the parabronchi, which are comparable to mammalian alveoli. Alveoli, however, are blind sacs, while parabronchi are loops that are connected to secondary bronchi at either end.

The majority of the larger cavities in your section of **avian lung**, **slide C-17**, are the smallest of the ducts: the **parabronchi (tertiary bronchi)**. **Atria (air vesicles)** are subdivisions of the walls of the parabronchi. The atria are lined by flattened cuboidal to squamous epithelial cells. **Air capillaries,** lined by a simple squamous epithelium, empty into the atria. There are smooth muscle cells in the walls of the parabronchi.

APPENDIX I

General Techniques for Light Microscopy

A. Principle

A preparation for the light microscope must be fixed, so that the structure does not decay in time; it must be relatively thin so that light can go through without too much distortion and absorption, and, finally, it must be appropriately stained. Unless special optical techniques are used, a thin section of biological material has little or no contrast if it is not stained.

B. Methods of procedure

1. Fixation

Fixatives have various effects: some separate out (basically precipitate) the solid from the aqueous phase. Others cross-link and/or stabilize specific components. Since some of the specimens are quite large, penetration of the fixative, and thus preservation, is enhanced by perfusing it through the vasculature.

Fixation is usually selective. For example, for the study of cellular relations and tissue patterns in large volumes, one would select Bouin's fluid, formalin, or formalin-Zenker's fluid because they penetrate rapidly without excessive shrinkage or swelling, and because many different stains could be used subsequently. Another example is the use of formalin, potassium bichromate, osmium solutions for fat droplets because lipids are minimally soluble in these fixatives. Another example is the use of freezing and drying to preserve chloride because its diffusion and loss are minimal after this treatment.

The following is a list of the main fixatives used in this course, and of their components.

	Saturated Picric Acid *(ml)*	Ethanol *(ml)*	Formalin (40% Formaldehyde) *(ml)*	Glacial Acetic Acid *(ml)*	Trichloracetic Acid *(ml)*	Mercuric Chloride *(g)*	Potassium Bichromate *(g)*	Sodium Chloride *(g)*	Distilled Water *(ml)*
Formaldehyde			10						90
Ethanol Acetic Acid		10-30		10					
Formalin-Zenker's Fluid			10	5		5	2.5		100
Susa			20		4	4.5		0.5	80
Formalin-Bichromate			20				3		100
Formalin-Mercuric Chloride			10			5			100

2. Staining

A stain is usually a colored organic compound which "has a special aptitude for being retained" by tissue elements. Such stains consist of two parts: a chromophore group which imparts color, and the auxochrome group which is responsible for making the dye soluble in water. The latter may be acidic or basic. For example, the chromophore or colored group of toluidine blue (see below) is positively charged because of the -NH_2 group. The amine is the auxochrome and is responsible for the positive charge of the dye and for making it water-soluble. The basic dye exists in the dry state as a chloride and in solution both dye and chloride ions are present. For another example, the chromophore or colored group of eosin Y (see below) is negatively charged because of the COO-. The carboxyl is the auxochrome, and is responsible for the negative charge of the dye and for making it water-soluble. The acid dye exists in the dry state as a sodium salt, and in solution both dye and sodium ions are present.

<u>Acidic and basic stains</u>. These terms may be confusing because the adjectives acidic and basic are not used in the conventional way. Ordinarily, an acidic substance liberates protons or hydrogen ions in aqueous solution or produces protons when reacting with certain metals or

ammonia; and a basic substance forms an excess of hydroxyl ions in an aqueous solution or binds protons and removes them from solution.

With stains, it is the nature of the auxochrome which determines whether the dye is acidic or basic. When the auxochrome group is the amino group ($-NH_2$), the amino group owes is basicity to its reaction with water:

When the auxochrome group is a carboxyl group (-COOH), the carboxyl group owes its acidity to its ability to dissociate and form hydrogen ions. Most basic stains form salts with chloride ions; most acidic dyes form salts with sodium ions. The solutions of basic dyes, being salts of weak bases and strong acids are acidic; the solutions of acidic dyes, being salts of weak acids and strong bases are basic. In other words, the terms acidic and basic when used to characterize stains refer to the charge on the auxochrome group (which is imparted to the chromophoric part of the dye) and not to the pH of the solution.

Some constituents of protoplasm are negatively charged (like nucleic acids) and combine preferentially with basic stains; these are called basophilic. Other constituents are positively charged and combine preferentially with acidic stains; these are acidophilic.

Here are some reasons for broadening the definition of the term stain.

a. Sudan and other stains for fat droplets are not really "stains" because they do not have an auxochrome group or appreciable solubility in water.

b. Silver, osmium and chromium solutions are used to "stain" mitochondria, fat droplets and the Golgi apparatus, and yet have no organic constituents.

c. Similarly, tests for chloride, phosphate-carbonate, iron, and calcium in cells and tissues can be made with exclusively inorganic reagents.

d. **Dehydration**: this involves immersing the specimen in a solution of increasing concentrations of solvent until all the water is substituted by the solvent (usually alcohol followed by toluene or acetone).

e. **Infiltration and embedding**: substitution of a solvent with embedding medium (paraffin, epoxy and other resins) and subsequent hardening of the embedding. Paraffin hardens by cooling to room temperature, resins require baking for various periods of time while they polymerize.

f. **Sectioning**: Paraffin embeddings allow sections of 5-10 micron thickness, plastic embeddings allow thinner sections, resulting in better images. The sections are picked up on glass slides. In the case of paraffin sections, the slide is covered with albumin; the paraffin is dissolved in toluene and the section is dehydrated before staining.

g. **Staining**: Staining involves several steps: immersion in the first stain (e.g., hematoxylin); wash and destain if needed, immersion in the second counter stain, wash. Staining is an art which requires close control of the results throughout. Paraffin sections accepts stains better than most plastic embeddings.

h. **Mounting**: The sections are dehydrated again, covered with a drop of mounting medium and a cover slip. The mounting medium protects the section and it has the same refractive index as glass. Without it, image distortion and loss of resolution occur.

Fixatives

Bouin's	Bouin's fluid is a saturated aqueous solution of picric acid containing 25 parts of formaldehyde (full strength) and 5 parts of glacial acetic acid.
Formalin bichr.	Orth's fluid is usually meant. This is 90 parts of an aqueous solution of potassium bichromate (30%) and sodium sulfate (10%) with 10 parts of 10% formalin.
Susa	The fixing fluid known as Susa is an aqueous solution containing mercuric chloride, 45 gm., sodium chloride, 5 gm., H_2O, 700 cc., 20% trichloroacetic acid, 100 cc., full strength formaldehyde, 200 cc., and glacial acetic acid, 40 cc. It is a "shot-gun" mixture.
Formalin	Formalin is usually used as a 10% aqueous solution of the full strength product which is a solution of the formaldehyde gas.
$HgCl_2$ Formalin	An aqueous solution of 10% formalin saturated with mercuric chloride.
Formalin Zenker	Formol. Zenker fluid, an aqueous solution of potassium bichromate (2.5%), sodium sulphate (1%), mercuric chloride (5%), and formaldehyde (5%).
Alcohol	Ethyl alcohol, usually absolute, sometimes containing 2% NH_4OH for subsequent staining by silver methods.
Osmic a	Osmic acid, used as a 1% or a 2% aqueous solution of OsO_4.

Special Various techniques for staining neuroglia, intracellular substances, cytoplasmic organoids and inclusions.

Bichromate Muller’s fluid is usually meant. This is an aqueous solution of potassium bichromate (30%) and sodium sulphate (10%).

Perfusion Perfusion of the fixing fluid through the circulatory system is indicated.

Embedding Media

Celloidin Concentrated ether-alcohol solutions of pyroxylin or of low viscosity nitrocellulose are used.

Paraffin Ordinary paraffin wax of suitable melting point is used.

Double Tissue is first embedded in rather dilute celloidin, hardened, and then celloidin-tissue block is embedded in paraffin.

Plastic Tissue is embedded in glycol methacrylate for thin sectioning (1-2 μ).

Stains

To visualize the various structures in fixed microscopic preparations a variety of stains is used; these fall into three principal categories:

a) Acidic stains, which color the cytoplasm (and nucleoli) of cells by forming complex colored salts with them;

b) Basic stains, which color the chromatin in the nuclei, Nissl material in nerve cells and certain other basophilic substances; and

c) Metallic impregnations, which have special uses in staining fine fibrils, cell boundaries, nerve cells, or which form insoluble compounds with fatty substances. Various combinations are used, and much depends upon the method of fixation as to the resultant stain.

Specific stains

A. Fuch. **Acid fuchsin**, used in 3% aqueous solution to stain cytoplasm and collagen; it is also a component of Mallory’s triple connective tissue stain.

Az. C.	**Azocarmine**, a cytoplasm stain used in 1% aqueous solution is a variant of Mallory's method.
Az. M.	**Mallory-azan**, the combination of Mallory's aniline-blue orange with azocarmine instead of acid fuchsin.
Azure B	A blue-green basic dye used to differentiate RNA and DNA.
Cajal	A method of impregnation with silver, essentially used as in photography, found particularly useful in staining nervous tissue. Various modifications have been used in your preparation.
Gold Cl	**Gold chloride** is used in impregnating nerve endings, and in toning and making permanent the silvered preparations.
Golgi	A method of impregnating with silver following fixation with potassium bichromate which leads to selective blackening of individual nerve cells with their processes.
H & E	**Hematoxylin and eosin** - the first is an important dye from dogwood combined with different mordants to stain chromatin. Eosin is a red acidic stain for cytoplasm, collagen, etc., often used in weak solution in alcohol.
I H & E	**Iron hematoxylin and eosin**. Hematoxylin combined with an iron mordant colors the finest granules. Nuclei are usually black or blue-black.
L. G.	**Light green**, an acid dye used as a counterstain for safranin.
M.	**Mallory's connective tissue stain** which uses aniline blue, acid fuchsin and orange G to color collagen blue, glia fibers red, elastic fibrils pink or yellow, nuclei red.
Mu. C.	**Mucicarmine**, a special stain used to color mucus red.
Os.	**Osmic acid** (osmium tetroxide) forms a deep brown or black insoluble compound with many fats, and can be used to fix delicate structures such as mitochondria and cilia.
Quadruple	**Kornhauser's orcein + alizarine + orange G + phosphotungstic and phosphomolybdic acids** in a quadruple stain for connective tissue. Elastic fibers stain red-brown; collagen reticulum or basement membranes stain green; nuclei stain blue or purple;

muscle fibers and cytoplasm stain violet or pink; myelin sheaths stain orange.

Res. fuch. — **Resorcin and fuchsin** is a stain for elastic fibers.

Silver — The nitrate in solution will stain particularly cell walls and the ground substance in connective tissue.

Thio. — **Thionin** is a basic dye used especially for Nissl bodies in nerve cells.

T. blu. — **Toluidine blue**, a form of methylene blue used like thionin.

Weig. — **Weigert's stain** for myelin sheaths resembles Weigert's but uses a different mordant and is quicker.

Wright — A popular blood stain, containing methylene blue eosin, staining the nuclei and metachromatic granules of white blood cells differentially.

APPENDIX II

ETYMOLOGY OR WHAT DOES THIS MEAN?

Selected Word List for Histology

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The vocabulary used in histology is based to a great extent on the utilization of words, word roots and prepositions derived from the Latin and Greek languages. They are seen in the English language as prefixes, suffixes or word stems. This list is intended to introduce the medical student to scientific vocabulary and to demonstrate how the terminology for histology is developed. A knowledge of some of the more frequently used Latin and Greek forms helps put the study of histology on scientific and rational basis as well as making it more readily and pleasurably mastered. This knowledge will also apply itself to the understanding of terminology used in other basic sciences and in the clinical field.

Rather than attempting to memorize the word list and definitions verbatim, repeated brief exposures associated with an active seeking of word definitions, either here or in the medical dictionary when a new word is encountered, should prove to be the most helpful for the majority of the students.

a, an — Greek prefix, indicating **without** or a **negative quality**, e.g. *acellular* - not containing cells; *amitosis* - cell duplication that does not go through the process of mitotic division.

ab — Latin prefix indicating **away from**, e.g., *abnormal, aberrant.*

acinus — Latin, a grape or berry, used in histology to designate the **sac-like arrangement of cells** forming glands, e.g., *pancreatic acini*, *parotid acini*.

ad — Latin preposition **to** or **towards**, sometimes seen as "af", e.g., *afferent lymphatics* - those lymphatic tubules that flow to a lymph node; *adrenal gland* - a gland next to the kidney.

adenos — Greek, **gland**, e.g., *adenohypophysis* - the glandular portion of the hypophysis; *adenoid* - the pharyngeal tonsil.

affin — Latin stem for **related** or **adjacent**, hence *argentaffin cells* - those which have granulation stainable with silver; *chromaffin cells* - those stainable with chromium salts.

alveolus — Latin, a small hollow pit, used histologically to indicate a **small sac-like dilation**, e.g., *pulmonary alveoli* - the microscopic sacs of the lung; *dental alveolus* - the cavity in bone in which a tooth is held.

anti — Greek preposition meaning **against**, e.g., *antigen, antibody*.

append — Latin, to **hang**, e.g., *appendix* - gross anatomical structure attached to the cecum (literally hanging from the cecum); *appendix testis* - a remnant of an embryonic tube attached to the superficial pole of the testis.

appos — Latin stem signifying **united, applied**, e.g., *appositional growth* - layering of cells on other preformed similar or dissimilar surfaces as in bone growth.

basis	Greek, **base** or **bottom**, e.g., _basalis layer_ of the endometrial lining of the uterus; _basilar_ region of a cell - that nearer to its supporting membrane.
blast	Greek for germ or bud, used in histology as a word stem indicating an **immature or precursor cell** type, e.g., _osteoblast_ - a bone-forming cell; _ameloblast_ - an enamel-forming cell.
calyx	Greek for **cup**, e.g., _renal cortex_ - cup-like structure into which collecting tubules deliver urine.
canaliculus	Latin, **tiny duct or tube**, e.g., _bile canaliculi_ - tiny bile-transporting ducts of the liver.
cardia	Greek for **heart**, e.g., _myocardium_ - the muscular portion of the heart; _pericardium_ - the connective tissue sac enclosing the heart; _cardia_ - the region of the stomach roughly near the heart.
chondro	Greek word stem for **grain, gristle, cartilage**, e.g., _chondroblast_ - cartilage-forming cell; _perichondrium_ - connective tissue surrounding cartilage; _mitochondria_ - submicroscopic cytoplasmic organelles.
chrom	Greek, **color**, e.g., _chromatin_ - the deeply staining material in a nucleus; _chromatolysis_ - dissolution and fragmentation of chromatin in a cell, or Nissl substance in a nerve cell.
clast	Greek word stem meaning **to break**, e.g., _osteoclast_ - a bone-destroying cell.
colliculus	Latin, a **mound** or **hill**, e.g., _seminal colliculus_ - an elevated ridge within the prostatic region of the urethra.
corona	Latin, **crown** or **wreath**, e.g., _corona radiata_ - an investment of granulosa cells around the ovum in a mature follicle; _coronary arteries_ - the arteries of the heart (they have a wreath-like distribution).
corpus	Latin noun for **body**, e.g., _corona cavernosus penis_ - a histologic structure in the penis in which blood may pool; _red corpuscle_ - the red cell of the blood; _corpus luteum_ of the ovary - yellow body.
cortex	Latin for **bark** or **shell**, e.g., _cortex_ of the kidney or adrenal gland.
crin	Greek system for signifying **to put out, expel**, hence this word base is used to formulate words applying to glandular function, e.g., _apocrine gland_- one whose cells release a portion of the cell itself as well as their product into the secretion versus _merocrine glands_ where the cell remains intact; _holocrine_.
crista	Latin, **crest**, e.g., the internal surface of mitochondria is composed of numerous shelf-like projections of the internal membrane called _cristae_.
cyte	Greek for **cell**, e.g., _cytology_ - the study of the structure and function of cells; _osteocyte_ - a bone cell.
decidua	Latin, indicating **to shed**, e.g., _deciduous teeth_ - the first set of teeth that precede the permanent set; _decidua_ - the lining of the gravid uterus which is shed at the time of delivery.
dendron	Greek, **tree**, e.g., _dendrite_ - the branched cytoplasmic processes which conduct impulses toward the cell body.

dens Latin, **tooth**, e.g., *dentin* - that portion of a tooth surrounding the pulp cavity; *dentinal tubules* - numerous small canals in the dentin.

desmos Greek, **chain, band**, e.g., *desmosome* - submicroscopic structure with a function of holding adjoining cells together.

dia Greek preposition **through**, e.g., *diapedesis* - the migration of white cells through a capillary wall; *diagnosis* - literally means "through knowledge".

didymis Greek for **testis** - used as a stem for words such as *epididymis* - a coiled tubule located on the superior pole of the testis.

duct Latin stem meaning **to lead**, presently used in sense of a tube or passageway, e.g., *ductus deferens*; *parotid duct*.

ectasis Greek, **extension, dilatation**, e.g., *atelectasis* - collapse of the lung (literally, imperfect expansion).

ekto Greek, **outside**, e.g., *ectoderm* - the external layer of the 3 germ cell layers; *exocrine gland* - one which releases its secretion outside of the tissue viaduct.

endo (end) Greek preposition for **in** or **within**, e.g. *endothelium* - the epithelial lining of blood vessels; *endoplasmic reticulum* - submicroscopic network present in the cytoplasm of cells.

epi Greek prefix meaning **on** or **upon**, e.g. *epidermis* - the external layer of the skin that overlies the dermis; *epinephrine* - a hormone produced by a tissue that anatomically rests on the kidney (nephros), i.e., the suprarenal gland.

erythro Greek for **red**, e.g., *erythrocyte* - red blood corpuscle; *erythroblast* - an immature red blood cell.

ex (ef) Latin preposition meaning **out** or **away from**, e.g., *efferent lymphatics* - those vessels carrying lymph out of a lymph node; *ductuli efferentes* of the testes - carry the sperm cells from the testes to the epididymis; *exogenous pigment* - that pigment which is produced elsewhere then incorporated by the cell.

folliculus Latin, **a small sac**, e.g., *follicles* of the thyroid gland - the thyroid acini; *liquor folliculi* - fluid present in ovarian follicles.

ganglion Greek for **swelling**, e.g., histologically used as a collection of nerve cell bodies outside of the central nervous system, e.g., *preaortic ganglia*.

genesis Greek for **origin**, hence, *histogenesis* and *organogenesis* are the development of a tissue or an organ, respectively.

germ Latin for **bud or sprout**, used in histology in sense of reproduction and development, e.g., *germ cells* - those cells concerned with reproduction of the organism; *germ layers* - 3 embryonic cellular layers which develop into the entire organism; *germinal center* of a lymphoid nodule.

glia Greek, **glue**, e.g., *neuroglia* - the connective tissue cells of the central nervous system; *microglia* - the smallest neuroglial cells.

hem, hemo Greek for **blood**, e.g., *hemoglobin* - the oxygen-carrying molecule in erythrocytes; *hematology* - the study of blood cells; *hemosiderin* - stainable tissue iron.

hepar	Greek, the **liver**, e.g., *hepatic parenchymal* cells - the glandular epithelium of the liver.
hetero	Greek, **other, different**, e.g., *heterogeneous* - composed of dissimilar elements.
hilus	Latin, that **part of the gland or tissue where blood vessels, nerves, etc.** enter.
histo	Greek stem for **tissue**, e.g., *histology*; *histiocyte* - one of the cells present in connective tissue.
homo	Greek root for **same, like, similar**, e.g., *homeostasis* - a state of balance.
hyper	Greek prefix indicating **more** or a **greater quantity**, e.g., *hyperchromatic*- staining more intensely than usual.
hypo	A prefix derived from the Greek meaning **under** or a **less quality**, e.g., *hypodermis* - the layer of areolar connective tissue subadjacent to the dermis; *hypochromasia* - a decreased staining affinity.
inter	Latin preposition **between**, e.g., *interlobular* ducts of various glands; *interstitial* cells of the testes are located in between the seminiferous tubules.
intercal (intra)	Latin preposition **into**, e.g., *intralobular* ducts of the pancreas.
iso	Greek, **equal, similar**, e.g., *anisocytosis* - inequality in cell size; *isotope*.
karyo	Greek for **nut** or **nucleus**, e.g., *karyolysis* - dissolution of the nucleus of a cell; *karyorrhexis* - rupture or fragmentation of a nucleus.
lacuna	Latin, **pit, depression,** e.g., *lacunae* of bone - cavities in bone containing the osteocytes.
lamella	Latin **diminutive form of lamina**, hence a **very thin, little plate**, e.g., *lamellae* of the endoplasmic reticulum - submicroscopic plates present in cytoplasm.
lamina	Latin for **thin plate**, e.g., *lamina propria* - the thin layer of connective tissue supporting the glands and epithelial lining of a mucosal layer.
leuko	Greek for **white**, e.g., *leukocyte* - a white blood cell; *leukemia* - a disease of the white blood cells.
luteus	Latin adjective for **yellow**, e.g., *corpus luteum* - the "yellow body" that is developed from an ovarian follicle after it has released its ovum; *lutein cells*- the cells of the corpus luteum.
lymph	Latin stem for **water**, e.g., *lymph* - interstitial fluid that has entered the lymphatic vascular channels; *lymphocytes* - cells present in lymph and lymph nodes.
macro	Greek, **large**, e.g., *macrophage* - a large phagocytic cell; *macroscopic*.
macula	Latin, **spot** or **stain**, e.g., *macula densa* - a collection of specialized cells in the distal convoluted tubule at the point where it lies adjacent to the afferent arteriole.
matrix	Latin for **site of generation and development**, used in histology as the ground work or frame of a tissue into which cells are placed, e.g., the *matrix* of dense connective tissue is

	essentially collagen fibers.
mediastinum	Latin meaning **"in the middle",** e.g., *mediastinum testes* - an area of fibrous tissue within the testes; the *mediastinum* of the chest - the central area of the thoracic cavity "between the two lungs".
medulla	Latin, the **inner layer or region**, e.g., *medulla* of a lymph node; *medullary rays* - groups of tubules extending from the medulla into the cortex in the kidney.
mega	Greek for **great**, e.g., *megakaryocyte* - a giant cell with a large multilobed nucleus that is found in the bone marrow and which produces platelets; *megaloblast* - an abnormal erythrocyte precursor that is (among other things) larger than normal.
meso	Greek stem meaning **middle** or **intermediate**, e.g., *mesoderm* - the middle layer of the 3 germinal layers; *mesothelium* - epithelium lining the peritoneal cavity.
mestos	Greek for **stuffed**, e.g., *mast cell* - a connective tissue cell containing large number of metachromatic cytoplasmic granules.
meta	Greek prefix used to indicate a **change** or **transformation**, e.g., *metachromasia* - the quality of a tissue to stain a color other than that of the stain used; *metamyelocyte* - a developing white cell that has progressed beyond the myelocyte stage.
metra (o)	Greek stem referring to the **uterus**, e.g., *myometrium* - the muscular layer of the uterus; *endometrium* - the mucosa of the uterus.
mikros	Greek, **small**, e.g., *microscopic, microscopy, micron*.
mitos	Greek, **thread**, e.g., *mitosis* - the process of cell division (descriptively, that time when the chromosomes become definitive and thread-like); *mitochondria* - the submicroscopic cytoplasmic organelles having a thread-like appearance.
morphe	Greek, **form or shape**, e.g., *morphology* - study of form and structure; *amorphous* - without a definite structure.
mu	Greek letter for **"m"** (written as μ); it is used as a measurement, *micron* - one thousandth of a millimeter. The erythrocyte is 7μ in diameter.
mucus	Latin for **slime**, used in histology in forms as *mucosa* - the glandular secretory layer of the intestine; *mucous* - (adj.) - containing or liberating a thick secretion; *mucin* - the chief constituent of mucus.
myel	Greek stem for **bone marrow**, also often taken to include the spinal cord, e.g., *myeloid cells* - white blood cells that develop in the marrow; *myeloblast*- an immature white blood cell; the *myelin sheath* of a nerve fiber.
myo (mys)	Greek root for **muscle**, e.g., *myocardium* - the muscular portion of the heart; *myenteric plexus* - a collection of nerve cell bodies between the muscular layers of the gut; *epimysium* - the layer of connective tissue surrounding a muscle.
nephos	Greek for **kidney**, e.g., *pronephros* - the early embryonic kidney; *nephron* - the unit structure of the kidney.
neuron	Greek for **nerve**. Used as a stem for many words relating nerve structure, e.g., *neuron* - a nerve cell; *epineurium* - the outer connective tissue layer surrounding a nerve; *neuroanatomy*.
odontos	Greek, **tooth**, e.g., *odontoblast* - cell associated with formation of dentin; *periodontal*

	membrane - one of the layers surrounding the root of a tooth.
oo	Word root from Greek "oon" meaning **egg**; hence this stem refers to the ovum, e.g., *oogonium* - the first stage in development of an ovum; *cumulus oophorus* - a collection of cells in which the ovum is embedded in a mature follicle.
osteon	Greek for **bone**, e.g., *osteocyte* - bone cell; *osteoid* - young bone not yet calcified.
ovum	Latin for **egg**, e.g., *ovulation* - the release of an egg from the *ovary* (the female gonad).
papilla	Latin, **nipple or little bump**, e.g., *lingual papillae* - tiny projections on the surface of the tongue, some of which contain taste buds; *papillary layer* of dermis - stratum of dermis containing connective tissue projections which penetrate into the epidermis.
para	Greek preposition, used to indicate position next to or a similarity, e.g., parachromatin - the stainable material in a nucleus other than the dark-staining chromatin; the parathyroid gland.
parenchyme	From Greek para and en (=in) and chei (to pour). The essential and proper tissue of an organ, e.g., liver parenchyme.
pars	Latin, part, e.g., pars distalis of the hypophysis; pars nervosa of the hypophysis.
penia	Greek for poverty, used as a suffix, e.g., cytopenia - a lack of cells; leukopenia - a decrease in the number of circulating white blood cells; thrombocytopenia - insufficient numbers of platelets or thrombocytes.
peri	Greek for around, encircling, e.g., periosteum - dense connective tissue layer that surrounds bone; perimysium - connective tissue investing small bundles of muscle fibers; periodontal membrane - dense connective tissue which produces firm connection between a tooth and bone; periodontia.
pes (ped)	Latin word root for foot, e.g., podocyte - renal cell with many cytoplasmic foot processes.
phago	Greek, to eat, e.g. phagocyte - a cell that engulfs foreign material; macrophage - a large phagocytic cell; esophagus - a portion of the intestinal tract leading to the stomach.
philos	Greek, loving, fond of, having affinity for, e.g., acidophilic - staining with acid dyes; argyrophilic fibers - those stainable with silver stains.
phobe	Greek, fear or resisting, e.g., chromophobe cells of the hypophysis - those that do not stain with usual stains.
phyl	Greek, tribe, race, e.g., phylogenesis of adrenal gland - comparative study of the development of the adrenal gland in various animal forms; monophyletic.
pino	Greek, to drink, e.g., pinocytosis - the process by which cells may engulf substances in solution by an involution of the cytoplasmic border and thus trap the material within a cytoplasmic structure called a pinocytic vesicle.
plasia	Greek stem indicating a moulding, e.g., hyperplasia - increased growth or development; anaplastic - lacking normal form.
plasma	Greek for anything formed or molded, used histologically for apparently structureless more or less amorphous structures, e.g., nucleoplasm - "nuclear sap"; cytoplasm; endoplasmic reticulum - a submicroscopic cytoplasmic network.
poikilo	Greek, varied, e.g., poikilocytosis - a variation in the shape of erythrocytes.

poly	Used as a prefix indicating many, e.g., polychromatic - staining more than one color; polyploid nuclei - those with more than the normal number of chromosomes.
portal	Porta means “the gate” in Latin - hence the portia hepatis refers to the areas where blood vessels and bileduct enter and leave the liver; a portal circulation is one, contrary to that usually seen, in which the same type of vessel (e.g., vein) drains away from and leads into a capillary or sinusoidal network (as in the hepatic portal system).
pre	Latin prefix indicating before or anterior, e.g., premolar - a tooth located anterior to a molar; the preclinical years of medical school.
pro	Greek preposition meaning before, e.g., prophase - the first stage of mitosis; promyelocyte - the immature white blood cell that develops into the myelocyte stage.
propria	Latin, one’s own special, e.g., lamina propria mucosae of digestive tract or urethra - the thin layer of connective tissue upon which the mucosa rests.
rectus	Latin, straight, e.g., tubuli recti - the straight tubules of the testes interposed between the coiled seminiferous and tubuli efferentes; erectile tissue of the penis - that spongy tissue which when engorged with blood results in swelling and erection.
rene	Latin for kidney, used as word root for referring to this organ, e.g., renal tubules; reniform - kidney-shaped; adrenal - next to the kidney.
rete	Latin noun for net, e.g., rete testis - a network of canals within the testes; reticulocyte - an erythrocyte that still has some retained cytoplasmic RNA which, when histologically demonstrated, presents an appearance resembling a network.
reticulum	Latin, the diminutive form of rete (a network), hence, a tiny network, e.g., reticular fiber - a type of fiber found in connective tissue especially in hematopoietic tissues where it forms much of the structural skeleton of the tissue; reticular cell - a primitive cell found in association with reticular fibers and whose cytoplasmic extensions join with one another to form a loose network.
sarcos	Greek for flesh, e.g., sarcoplasm - the material between the myofibrils in the cytoplasm of a muscle cell; sarcolemma - the delicate membrane investing single muscle fibers.
septum	Latin, partition, e.g., alveolar septa - the walls of the pulmonary alveoli; septal cells - macrophages present in alveolar septa.
serous	Latin for “whey”, watery, e.g., serous gland - one which elaborates as a watery secretion; blood serum ; a serous membrane.

sinus Latin, **a bay of the sea**, e.g., _hepatic sinusoids_ -small irregular blood vascular channels interposed between liver cells.

soma Greek for **body**, e.g., _somatic cells_ - cells other than the germinal cells; _microsomes_ - submicroscopic cytoplasmic particles; _ribosomes_ - microsomes containing ribose nucleoprotein.

squam Latin, **scaly, flat**, e.g., _squamous epithelium_ - thin, plate-like epithelium.

stereo Latin, solid, frequently used to denote **"having three dimensions",** e.g., _stereocilia_ - non-motile cilia-like projections of the cytoplasm.

stratum Latin noun for **layer**, e.g., _stratum corneum_ of the skin - the cornified layer; _stratified_ (adj.) - layered.

sub Latin, **under or below**, e.g., _submucosa_ - the layer of the intestinal tract wall deep to the mucosa; _sublingual gland_.

supra Latin preposition **above or superior**, e.g., _suprarenal gland_ - the adrenal gland - anatomically located on the superior pole of the kidney.

syn (m) Greek stem for **together**, e.g., _syncytium_ - an aggregation of cells without definite cell boundaries; _synapsis_ - the paring and union of homologous chromosomes at the start of meiosis.

tela Latin, **web or woven structure** - used histologically as a layer, e.g., _tela submucosa_ - the layer deep to the mucosa.

telo Greek stem indicating **far, distant, the end**, e.g., _telophase_ - the last stage of mitosis.

theca Greek noun - **sheath or covering**, e.g., _theca externa_ and _theca interna_ - layers of cells that envelop the developing ovum.

thrombos Greek, **clot**, e.g., _thrombocyte_ - circulating cytoplasmic fragments of megakaryocytes having functions associated with clotting blood.

trans Latin preposition **across**, e.g., _transition epithelium_ - epithelium intermediate in appearance between squamous and columnar epithelium; _translucent_..