LAB MANUAL - MICROSCOPY LAB
BMD 415, Microscopic Anatomy, Fall 2004

INTRODUCTION: The microscopy lab is a tissue recognition exercise. We will observe examples of almost all organs and tissues in the human body and note their structural specializations and characteristic features. The microscopic structure often helps to explain how an organ works, and the normal appearance must be understood to be able to recognize disease states. While microscopy is a very important tool in biomedical sciences, you must also be aware of both its limitations and its potential. By examining actual slides rather than computer images, you will become more knowledgeable of variations in structure, fixation, dissection, sectioning, staining, and coverslipping that will contribute to or detract from the overall image quality. You will find that microscopy is a valuable tool and that its usefulness to you will be enhanced by an understanding of the processes behind the technique.

STUDENT SLIDE COLLECTIONS: Please handle your slides with care; they represent a large investment of departmental resources and are difficult to replace. Do not remove them from the lab. The collection contains both human and animal specimens because human material is usually difficult to fix adequately, and because most animal tissue structures are very similar to human. Unless otherwise noted, the slides are paraffin sections stained with hematoxylin and eosin. You may exchange slides in your collection for slides in the reserve boxes; see Dr. Itaya.

For all microscopy labs you are responsible for the structures listed for each slide. Key elements are underlined. There may be variations between slide sets. If you have a slide missing, or if your slide does not have all the structures described in your manual, borrow the slide from another student. A 5-headed microscope is available for group study in the back hallway.

BMD 415, Microscopic Anatomy, Fall 2004

	Lecture / Chapter	Lab
T 8/24	Course Introduction	1. Introduction, Check in,
R 8/26	Tissue Processing, EM, IHC / 1	Video, Tissue Processing Intro
T 8/31	Review Cells / 2-3, Epithelium / 4	2. Epithelium
R 9/2	Connective & Adipose Tissue / 5-6	3. Connective & Adipose Tiss.
T 9/7	Cartilage & Bone / 7-8, practice quiz	4. Cartilage & Bone
R 9/9	Nervous Tissue / 9	5. Nervous Tissue
T 9/14	Muscle Tissue / 10	6. Muscle Tissue
R 9/16	Muscle Tissue / 10	6. Muscle Tissue
T 9/21	REVIEW	Practice Exam
R 9/23	Lecture Exam 1 (50 pts)	Lab Exam 1 (50 pts)
T 9/28	Circulatory System / 11	7. Circulatory System
R 9/30	Blood Cells / 12	8. Blood
T 10/5	Immune System / 14	9. Immune System
R 10/7	Digestive System / 15	10. Digestive System
T 10/12	Digestive System / 15-16	11. Access. Digestive Organs
R 10/14	Respiratory System / 17	12. Respiratory System
T 10/19	Skin / 18	13. Skin
R 10/21	REVIEW	Practice Exam
T 10/26	Lecture Exam 2 (50 pts)	Lab Exam 2 (50 pts)
R 10/28	Urinary System / 19	14. Urinary System
T 11/2	Endocrine System / 20,21	15. Endocrine System
R 11/4	Male Reproductive System / 22	16. Male Reproductive System
T 11/9	Female Reproductive System / 23	17. Female Reproductive Sys.
R 11/11	Eye / 24	18. Eye
T 11/16	Ear / 25	19. Ear
R 11/18	REVIEW	REVIEW
T 11/23	Optional Presentations	Finish slide preps
R 11/25	HOLIDAY	HOLIDAY
T 11/30	Optional Presentations	REVIEW
R 12/2	REVIEW (video lab slides) Course Eval.	Practice Exam
T 12/7	Lab Exam 3 (video exam, 25 pts-old)	Lab Exam 4 (50 pts-new)
R 12/14	Final Exam: 10:30-12:30 (75 pts = 25 old+50 new))	

Reminder: Lab is closed every Monday for Physiology Lab class, exceptions will be announced
Video lab exam will be based on slides from the histology links listed at the course website.

LAB 1: INTRODUCTION, EQUIPMENT CHECK OUT, VIDEO, TISSUE PROCESSING

Review the lab schedule and syllabus. Fill out the Lab Equipment Check Out Form, sign, and turn in. View the videotape on the use of the microscope and familiarize yourself with your microscope. See demonstrations of poor slides. Check out the 5-headed microscope in the back hallway and extra microscopes in room 6128. Plan, schedule, and sign up for tissue processing labs. The lab instructor for tissue processing will be Lu Brown.

Remember: You are responsible for all electron micrographs in your text book and in other class resources.

LAB 2: EPITHELIAL TISSUE

Slide 45: Cardioesophageal junction, Rat

Observe the transition from stratified squamous keratinized epithelium (rat esophagus) to simple columnar epithelium (stomach). In the stomach, simple tubular glands (gastric glands) open onto the epithelial surface. Deep to the epithelium and surrounding the glands is the lamina propria. Compare cross, longitudinal, and oblique sections of gastric glands.

Slide 62: Larynx, Rat

This is a cross section through the esophagus and larynx, which is surrounded by laryngeal cartilage. Lining the laryngeal cavity is pseudostratified columnar ciliated epithelium. Observe the location of the esophagus.

Slide 49: Small intestine, Rat

The small intestine is lined by a simple columnar epithelium with a brush border made of microvilli at the apical surface. Interspersed among the epithelial cells are goblet cells, unicellular mucous glands. Deep to the epithelial cells is the lamina propria. Note the polarity of the epithelial cells with regard to placement of the nuclei, cytoplasm, and apical and basal surfaces. Between villi are the openings of intestinal glands (crypts of Lieberkuhn), which are simple tubular glands. Compare cross sections of villi with intestinal glands.

Slide 51: Large intestine, Rat

The large intestine is also lined by a simple columnar epithelium with numerous goblet cells. The goblet cells also extend into the intestinal glands, which are simple tubular glands. In this preparation, terminal bars, which are the intercellular junctional specializations of tight junctions and zonula adherens, can be seen as tiny, darkly staining dots between epithelial cells near their apical surface.

Slide 58: Pancreas, Human

Most of the tissue consists of exocrine acinar cells, with secretory granules in the apical region. Among the acinar cells are ducts, lined by columnar epithelium. Lighter staining clusters of cells are islets of Langerhans, the endocrine portion of the pancreas. Within areas of connective tissue are pancreatic ducts, lined by simple cuboidal/columnar epithelium.

Slide 64: Lung, Rat

Look for bronchioles, which are the larger air passageways. They are lined by pseudostratified columnar ciliated epithelium (rare) or simple columnar ciliated epithelium. Examples of simple squamous epithelium can be seen in the endothelium of blood vessels.

Slide 66: Thick skin, Human

The epidermis is composed of stratified squamous epithelium that is keratinized. Within the layers of the epidermis, the cells just above the basal layer make up the stratum spinosum. Look for their "spiny" outlines, which are caused by numerous desmosomes maintaining attachment between cells which have shrunken apart in processing.

Slide 69: Kidney, Rat

The cortex of the kidney is composed of spherical glomeruli surrounded by tubules. The epithelium lining the tubules is simple cuboidal. Proximal convoluted tubules, identifiable by their relatively dark staining, have striations at their basal surfaces (basal striations). These are interdigitations with neighboring epithelial cells which increase the surface area, and they mark the location of Na^{+}/K^{+} ATPase, the sodium pump.

Slide 70, 71: Urinary bladder, Pig, and Ureter, Human

The urinary bladder and ureter are lined by transitional epithelium, which has the property of being able to expand and contract while maintaining a barrier to diffusion of urine into subepithelial tissues.

Slide 85, Testis, Rat

In the lumen of some of the seminiferous tubules are spermatozoa with long, wavy tails. The tails are examples of flagella. The lining epithelium is a complex Pseudostratified.

LAB 3: CONNECTIVE TISSUE and ADIPOSE TISSUE

Be able to recognize the different types of connective tissue, connective tissue fibers, and cartilage.

Mesenchyme

Slide 83: Umbilicus, Human

This cross section contains two arteries and a vein. The connective tissue around the blood vessels is called Wharton's jelly; it is mucous connective tissue, a type of mesenchyme, which contains an abundance of watery ground substance. The cells are mainly mesenchymal cells.

Loose Connective Tissue (see also cardiac muscle & tongue)

Slides 45, 47, 49: Gastrointestinal system, Rat; 86: Epididymis, Rat

In the gastrointestinal system examine the lamina propria of several slides for examples of loose connective tissue, or areolar tissue, which is also located around all blood vessels. Examine the connective tissue between the epididymis tubules. You will not be able to identify specific components, but be aware that collagen, elastic, and reticular fibers are present, as well as fibroblasts, macrophages, lymphocytes, mast cells, and white blood cells. For comparison, dense irregular connective tissue is located in the submucosa.

Adipose Tissue
Slides 36: Thymus, Human; 51: Large Intestine, Rat

The fat cells can be seen as a thin rim of cytoplasm and a peripheral nucleus around a large, central fat droplet, which has been washed out during tissue processing.

Dense Irregular Connective Tissue
Slides 73/74: Adrenal Gland, Human; 93, Eye, Human

The capsule and stroma of many organs are made up of dense irregular connective tissue. In the eye, the sclera is composed of dense irregular connective tissue. Observe the fibroblast nuclei and the fibrous matrix. Notice the low proportion of cells compared to loose connective tissue.

Review different types of connective tissue:
Slide 66: Skin, Human

In the skin, loose connective tissue is located in the papillary layer of the dermis, the layer at the junction between the epidermis and the dermis. The reticular layer of the dermis, between the papillary layer and the subcutaneous tissue, is made up of dense irregular connective tissue. Adipose tissue can be seen in the subcutaneous layer.

Dense Regular Connective Tissue
Slides 1, Tendon, Human

Dense regular connective tissue is characterized by parallel arrangement of collagen fibers and fibroblasts. This organization produces resistance and high resistance to tension in one direction. Dense regular connective tissue is found in ligaments, also.

Elastic Fibers
Slide 27: Aorta, Pig, Orcein Stain

The wall of the aorta consists principally of elastic fibers and smooth muscle fibers. The elastic fibers are light brown stripes interspersed among the nuclei of the muscle fibers.

The bulk of the wall of the aorta in cross section is made up of the muscular layer, which is composed mainly of elastic fibers and smooth muscle cells. The elastic fibers appear as wavy light brown lines oriented in a circular direction around the lumen of the aorta.

Reticular Fibers
Slide 35: Spleen, Silver stain, Rat

Reticular fibers make up a "skeletal" support system for many lymphoid organs, on which are anchored reticular cells, macrophages, and lymphocytes. To be seen, however, reticular fibers must be specially stained with silver preparations. Notice their thin, branching structure, organized into a network. This section has been counterstained with hematoxylin and eosin.

Reticular Fibers
Slide 56: Liver, Silver Stain, Rat

As in the spleen, branched reticular fibers form a network that helps to maintain the organization of the liver tissue. Look for black reticular fibers along hepatocytes and around blood vessels. This section has been counterstained with hematoxylin and eosin.

LAB 4: CARTILAGE AND BONE

Hyaline Cartilage
Slide 60: Trachea, Human

In the wall of the trachea are "C" shaped rings made up of hyaline cartilage. Observe the chondrocytes in the lacunae, and the perichondrium around the cartilage. The staining immediately adjacent to lacunae is territorial matrix which is rich in glycosaminoglycans which are basophilic. The lighter staining between lacunae is the interterritorial matrix.

Elastic Cartilage
Slide 4: External ear, Human

This is a section through the external ear (lobe). The superficial surface is covered by thin skin (stratified squamous, keratinized epithelium) with hair follicles. Deep to the skin is the subcutaneous layer with loose connective tissue along with blood vessels and nerves. At the center of the sample is elastic cartilage. In this H&E preparation, elastic cartilage appears very similar to hyaline cartilage with a perichondrium, chondrocytes, and matrix. In this preparation, the identity of the type of cartilage must be based mainly on its location.

Slide 6: Ground bone, Human

This is a ground cross section of compact bone. The slide was prepared by grinding a piece of dried bone to a thinness that can be transilluminated; thus there are no cells, blood vessels, or any other soft tissue. Observe the organization of the osteon, or Haversian system, including the Haversian canal, Volkmann's canal, lamellae, canaliculi, lacunae, and interstitial lamellae. Notice the overlapping pattern of new and old osteons.

Slides 7 & 8: Decalcified bone, Rat

These sections contain compact bone surrounding spongy bone in a red marrow, where hematopoiesis is occurring.

The compact bone is surrounded by periosteum, consisting of collagen fibers and fibroblasts. An inner layer of flattened cells make up osteoprogenitor cells. There are also skeletal muscle fibers, a few of which are attached to the bone.

In the compact bony tissue are osteocytes, located in lacunae, and blood vessels, but Haversian systems are not visible. The inner surface of the compact bone is lined by endosteum, composed of osteoprogenitor cells.

The spongy bone is composed of bony spicules which have a microscopic structure and organization very similar to compact bone. In the marrow cavity are developing blood cells, including megarakaryocytes, which are large, multinucleated, round cells which produce platelets.

Osteoclasts, if present, will be large, multinucleated, irregularly shaped cells located in depressions along the surface of bone (Howship's lacunae).

Slide 9: Endochondral bone formation, Rat

At low power, identify the epiphyseal plate, the primary ossification center, the periosteum, the diaphysis with its bony collar, the spongy bone of the red marrow cavity, and the epiphysis. At higher magnification, observe the chondrocytes, hyaline cartilage, osteoblasts, osteocytes, and spongy bone. Notice the darkly stained nuclei of all the cells, particularly the osteocytes. This tissue has been fixed, decalcified, sectioned, and stained, in contrast to the ground bone section.

Bone growth occurs at the epiphyseal plate, where cartilage grows and also develops into bone. There are five zones of development: 1) the resting zone, composed of hyaline cartilage; 2) the proliferative zone, where chondrocytes multiply by mitosis; 3) the hypertrophic cartilaginous zone where chondrocytes enlarge; 4) the calcified cartilage zone, where the cartilage matrix becomes calcified and chondrocytes die; and 5) the zone of ossification, where osteoprogenitor cells invade and become osteoblasts, which deposit matrix over the calcified cartilage matrix.

LAB 5: NERVE TISSUE

Slide 10: Peripheral nerve, Rat, Osmium stain

Osmium is a fixative and stain which fixes lipids, unlike routine formaldehyde fixation. In addition, the rat eye specimen was embedded in plastic (epoxy) to be able to cut 1 μm sections. Along one edge of the section are darkly stained circular or elongated profiles. These are myelinated axons in cross section; occasionally there are longitudinal sections. Immediately surrounding the nerve is loose connective tissue, and around the connective tissue are skeletal muscle fibers of the extraocular muscles. At the opposite edge of the section is a cross section of the posterior eye ball.

Slide 40: Tongue, Human

Among the striated muscle fibers making up the bulk of the tongue are numerous bundles of myelinated axons, cut in various orientations. Compare this formaldehyde-fixed, paraffin-embedded section to slide 10.

Slide 14: Golgi stain, Rat CNS

The Golgi stain selectively and randomly impregnates neuronal cell bodies and processes, blood vessels, and neuroglia with a silver precipitate. In a fortuitous Golgi stain, you will see a black nerve cell body with all of its processes, against a pale yellow background, which contains unimpregnated neurons. This permits observation of neuronal structure that no other technique allows. The sections are 100 μm thick, so focusing up and down is important to follow all structures. Look for dendritic arborization, and dendritic spines, which are usually postsynaptic sites. Isolated axons may be visible as well, however, they are usually cut during the process of sectioning.

Slide 11: Cerebral cortex, Human

Look for an untrimmed surface of the tissue; this should be a pial surface and the cortical layers will be parallel to this surface. Layer I has only nerve processes. Layers II and III are indistinguishable and are characterized by small neurons. Layers IV-VI contain pyramidal neurons.

Deep to layer VI is the white matter, which is devoid of neuronal cell bodies and contains mainly myelinated axons and glial cells which are noticeably smaller than neighboring nerve cell bodies.

Slide 13: Geniculate ganglion, Decalcified preparation, Rat

The geniculate ganglion contains the sensory neurons of the facial nerve, and it is located in the temporal bone. In this preparation, the geniculate ganglion is located along the periphery of the section.

The structure of the ganglion is composed of neurons, satellite cells, and myelinated fibers. Because the sensory neurons are unipolar, they are large cells which are round to oval in shape. They have a prominent, centrally located nucleus and nucleolus, and basophilic Nissl bodies in the cytoplasm. Surrounding the neurons are satellite cells, or supporting cells. The eosinophilic areas between neurons are occupied by myelinated axons, with accompanying Schwann cells and blood vessels.

While much of the fatty myelin washed out during the clearing step of processing, you can still find cross sections of axons surrounded by the remnants of a myelin sheath, or longitudinal sections of myelinated axons with nodes of Ranvier.

Slide 15: Cerebellum, Human

This cross section through several lobules demonstrates the highly organized cellular layers of the cerebellum. The grey matter is composed of a pink staining molecular layer which has myelinated fibers and glial cells mainly, with scattered neuronal cell bodies. The dark purple layer is the granular cell layer, packed with small granular neurons. In between the two layers, look for profiles of Purkinje cells. These are large neurons with an extensive dendritic arborization in the molecular layer. Deep to the granular layer is the white matter.

Slide 16: Cerebellum, Rat

The rat cerebellum is organized like a miniature human cerebellum. Notice that the rat Purkinje cells are much more numerous than in the human and form a nearly continuous row. The cerebellum is attached to the brainstem on this slide; look for nerve cell bodies, white matter, and blood vessels in the pons. In the crevice between brainstem and cerebellum look for choroid plexus. It is a thin, convoluted membrane lining the fourth ventricle, composed of pia mater, ependyma, and numerous capillaries, with the function of secreting cerebrospinal fluid.

Slide 17: Spinal cord, Human

At low power, or without the microscope, identify the H-shaped grey matter, with the dorsal and ventral horns, and the surrounding white matter. The ventral horn can be identified at low power by the presence of very large, multipolar motor neurons. The neuronal perikarya in the dorsal horn are much smaller. Examine the white matter. Look for cross sections of myelinated axons. In this preparation, the myelin has been dissolved away, and the axons have shrunk. Yellow to brown granules in cells are generally lipofuscin granules, or residual lysosomes.

Slide 18: Spinal cord, Decalcified preparation, Rat

This is a decalcified preparation of the rat spinal cord and surrounding vertebra. At low/medium power, identify the ventral and dorsal horns, the funiculi, the central canal, dorsal and ventral roots, dorsal root ganglia, the dura mater, arachnoid (not complete), the pia mater, and the

anterior spinal artery. Lining the central canal are ependymal cells, forming a simple columnar epithelium.

At medium/high power, compare the sizes and shapes of neuroglial cells (usually smaller nuclei) with the different neurons (larger nuclei with basophilic cytoplasm). Note how the shape of multipolar motor neurons in the ventral horn compared to unipolar sensory neurons of the dorsal root ganglion. You should observe the same structures in the dorsal root ganglion that you saw in the geniculate ganglion. Look for Nissl bodies, and dura mater, arachnoid, and pia mater.

Slide 20: Sympathetic ganglion, Human

This is a section of a superior cervical ganglion, from the sympathetic chain ganglia. The postganglionic neurons are multipolar, so their shape is more irregular than unipolar, sensory neurons. The neurons have a prominent nucleus and nucleolus, and basophilic Nissl bodies in the cytoplasm. Compared to sensory ganglia, there are fewer satellite cells and myelinated axons, and the neurons are more homogeneously located within the ganglion. Look for orange/brown lipofuscin granules in the cytoplasm of some nerve cell bodies.

Differences are subtle between unipolar neurons and sympathetic postganglionic neurons and you will not be expected to differentiate the two in this course.

Slide 45: Cardioesophageal Junction, Rat

Surrounding the stomach are two layers of smooth muscle. Look between the layers for large, basophilic cell bodies. These belong to the myenteric plexus, part of the autonomic nervous system. They are postganglionic, parasympathetic neurons which are part of the enteric nervous system.

Slide 95: Optic papilla, Rat/Human

The optic nerve is composed of myelinated axons, cut longitudinally in this section. Around the optic nerve are pia mater, subarachnoid space, and arachnoid and dura mater, usually fused.

LAB 6: MUSCLE TISSUE

Slide 21 deltoid, 40 tongue: Skeletal muscle, Human

Scan the section at low power first and find areas where fibers are cut longitudinally. At higher power, you should be able to see the A, I, and H bands. Notice the peripherally located nuclei.

Slides 23, 96: Skeletal muscle, Rat eye

This is an osmium treated, Toluidine blue stained, 1 μm plastic section of rat eye, including some extraocular muscle fibers. Find a longitudinal section of muscle fibers and observe the enhanced clarity of the muscle striations (due to the thinness of the section); try to identify the A, I, and H bands. Many sections are cross/oblique cuts. The dark staining structures in the sarcoplasm are mitochondria.

Slide 28: Cardiac muscle, Human

This specimen extends through the cardiac wall. On one side there is visceral pericardium (epicardium) which includes serous epithelium, fat tissue, and blood vessels. At low power, note bundles of cardiac muscle fibers separated by connective tissue and blood vessels. The bundles of muscle fibers create an irregular inner surface of the heart, the trabeculae carnae. The heart chamber has a thin lining, the endocardium, composed mainly of endothelium. In addition, on this specimen the endothelium has a fuzzy, eosinophilic, extracellular coating of unidentified origin.

Use medium power (10 x objective) to examine cardiac muscle fibers. The orientation of the fibers is in all directions. Note the size and shape of cardiac fibers, the eosinophilic cytoplasm and centrally located, basophilic nucleus. Look for an area of longitudinal sections to examine at high power.

Structural characteristics to look for at high power include: striations, branching of fibers, lipofuscin granules (appearing yellow-brown), and intercalated disks (white or dark lines perpendicular to the long axis of the fiber). The orientation of some fibers clearly shows their quadrilateral shape. Between fibers there are small, fusiform nuclei of fibroblasts and light pink connective tissue fibers.

Be able to recognize cardiac muscle and differentiate it from skeletal and smooth muscle.

Slide 29: Cardiac muscle, Rat

Use the same approach to view the rat cardiac muscle fibers. Since the rat fibers are less spread out, branching is difficult to see. Find an area of cardiac fibers cut in cross section and notice the centrally located nuclei. Striations and intercalated disks are difficult to see in this preparation.

Smooth Muscle, Cross & Longitudinal Sections
Slide 48, Small intestine, Human; 88, Prostate, Human

Notice the numerous elongated nuclei and the lack of striations in the smooth muscle layers. Where smooth fibers are cut in cross section, observe the centrally located nuclei and the varying diameter of the fibers, depending on the level of the section through each fiber. Compare the morphology with tendon and peripheral nerve fibers.

Around the glandular tissue in the prostate are scattered smooth muscle fibers in all orientations.

LAB 7: CIRCULATORY SYSTEM

Slide 36: Thymus, Human; 42: Lip (medium arteries); 23: capillaries among muscle fibers; 40 Tongue; 53, Salivary gland; 70 Ureter; 51 Large Intestine (see epiploic appendages)

Scan the section at low power. Traversing the fatty tissue of the thymus are several pairs of arterioles and venules, and small muscular arteries and veins. Look for the components of the tunica intima, media, and adventitia in the different sized vessels, e.g., endothelium, subendothelium, internal elastic membrane, smooth muscle fibers, and loose connective tissue.

Slide 87: Ductus deferens, Human; 43 Esophagus (endothelium); 71: Bladder (arteries)

Accompanying the ductus deferens in the spermatic cord are several large veins, seen mainly in cross section. In the esophagus and bladder are small and medium arteries and veins. Look for valves in the veins (slide 43).

Slide 26-27: Aorta, Pig

This is a section of a dissected piece of the wall of the aorta. Identify the tunica intima, tunica media, and adventitia. The tunica media is composed of smooth muscle fibers and elastic fibers. Note the large diameter compared to other arteries.

Look for examples of arteries, veins, and capillaries in all tissues.

Slide 55: Liver

Between cords of hepatocytes are liver sinusoids, which are like large, leaky capillaries, incompletely lined by endothelium.

LAB 8: BLOOD

Slide 31: Peripheral blood smear, Human

Examine the edges of the smear, or areas where there is an even monolayer of cells with the 40x objective. Observe the erythrocytes, and neutrophils, and try to find eosinophils, lymphocytes, and monocytes. Consider yourself extremely lucky if you find a basophil; if you do, share your finding with the rest of the class.

Slide 32: Blood smear, Human, sickle cell anemia

There are few sickled red blood cells present because the blood was drawn from a patient between crises. Look for the same cells as in the normal sample, particularly eosinophils. Exposure to air and/or damage causes red blood cells to become crenated. They look like spheres with spikes.

Slide 30: Blood smear, Human, malaria

Malaria is caused by a parasite, *Plasmodium* spp., carried by *Anopheles* spp. mosquitoes. In the human host the parasite is found primarily inside of the red blood cells (RBC). The parasites invade rbc's and reproduce, eventually disrupting the rbc and releasing new parasites. Infected rbc's can be identified by basophilic staining within the cytoplasm.

LAB 9: IMMUNE SYSTEM

Slide 34: Spleen, Human

Several structures can be seen at low power: trabeculae made of dense irregular connective tissue, trabecular arteries and veins, running in trabeculae, white pulp composed of lymphocytes around a central artery, and red pulp which occupies the rest of the tissue volume. The white pulp forms PALS (periarteriolar lymphatic sheaths) composed of T cells, and lymph follicles, consisting of spherical clusters of B lymphocytes. Around both PALS and lymph follicles is a marginal zone where lymphocytes migrate out of capillaries. Examine the red pulp at high magnification and observe the splenic sinuses and the splenic cords between the sinuses. Look for macrophages containing residual bodies. In this and the next section there may be knife marks.

Slide 35: Spleen, Rat, Silver stain

The reticular fibers, which make up the stroma can be seen with silver stain. The location of trabeculae is discernible as is the structural support for the parenchyma.

Slide 37: Thymus, Rat

At low power, the capsule is visible, along with a few trabeculae. The trabeculae divide the gland into lobules, each with a clear cortex and medulla. What type of lymphocytes are these?

Slide 36: Thymus, Human

Little is left of the lymphoid tissue in this specimen from an aged individual which has undergone involution; most of the thymus consists of fat. In the small islands of lymphocytes remaining, look for Hassall's corpuscles (degenerated reticular cells).

Slide 38: Tonsil, Human

Study the structure of the tonsil at low power, e.g., the type of overlying epithelium, the number of crypts, the nodules, and the basal area with connective tissue and trabeculae. Based on your observations and using information from your text and lecture notes, you should be able to identify the specific tonsil. Notice also the germinal centers of the nodules, and the differing extent of lymphocytic infiltration of the epithelium (compare to figure 14-22, p. 280 in Junqueira). [Answer at end of lab.]

Slide 33: Lymph gland (lymph node), Human

At low power note the capsule and the subcapsular sinus. There are few trabeculae present, but look for trabecular sinuses. Note the nodules with germinal centers in the outer cortex. Although the cortex and medulla are difficult to differentiate in this section, B lymphocytes are generally located in the outer cortex, while the inner cortex has few nodules and contains T lymphocytes. The central medulla is made up of medullary cords, medullary sinuses, and trabeculae with blood vessels. Trace the path of lymph through the node.

Slide 77: Oviduct

Examine the area of the fimbria of the oviduct for lymphatics and blood vessels.

Slide 38: palatine tonsil

LAB 10: DIGESTIVE SYSTEM - GASTROINTESTINAL TRACT

Slide 42: Lip, Human

There is a transition from mucous membrane, which lines the oral cavity, to skin, on the outer surface. Mucous membrane is composed of stratified squamous nonkeratinized epithelium, with seromucous glands in the lamina propria and submucosa. Serous cells are basophilic with a ~cuboidal shape and spherical nuclei. Mucous cells are larger with a pyramidal to columnar shape, clear cytoplasm, and a flattened nucleus at the base of the cell. Ducts have a prominent lumen and are lined by simple-stratified cuboidal epithelium. There are numerous blood vessels in the vicinity, also. Deep to the submucosa are skeletal muscle fibers in cross section, part of the orbicularis oris muscle.

Slide 39, Tooth, Human

Decalcified paraffin section

Low Power: There are two specimens in our collection, an incisor and a molar. At low power you see mainly the eosinophilic dentin because the enamel has dissolved away in the decalcifying process (why?). The enamel used to cover the apical surface of the dentin, making up the crown. The dentin surrounds the pulp cavity, which contains loose connective tissue. At the level of the tooth root the dentin is surrounded by cellular tissue.

Medium-High Power: At the junction between dentin and pulp cavity is a layer of odontoblasts, elongated cells with a basophilic nucleus and an apical odontoblast process that extends the width of the dentin. The odontoblast process is located in dentinal tubules that are visible as fine, parallel lines through the dentin.

The remainder of the pulp cavity is occupied by loose connective tissue with fibroblasts and blood vessels.

At the neck of the tooth the cementum is visible as a slightly darker staining layer covering the outer surface of the dentin at the root. The cellular layer around the cementum is the periodontal ligament, which contains collagen fibers anchored in the cementum and in the alveolar bone.

Slide 40: Tongue, Human

This sample was taken from the dorsolateral surface near the tip of the tongue. The mucous membrane covering is similar to that seen on the inner surface of the lip. The muscle fibers have all of the characteristics of skeletal muscle, and the fibers run the three different planes, which is diagnostic of tongue.

Slide 41: Taste Buds, Human

Low Power: Circumvallate papillae from the dorsal surface of the posterior tongue were dissected and processed for paraffin sections. This specimen was cut at an oblique angle that results in papillae cut into several islands of tissue. The surfaces are covered with stratified squamous nonkeratinized epithelium.

There are numerous indentations of lamina propria into the epithelium. The lamina propria is highly vascular loose connective tissue.

Medium-High Power: Focus on the epithelium of the largest "island," which is a circumvallate papilla. Where the papilla lies adjacent to a narrow groove, examine the light staining clusters of cells (taste buds) embedded in the epithelium. The nuclei of the taste bud cells are spherical and stand out from the epithelial cells and the lamina propria cells. The taste bud extends to the epithelial surface where it forms a taste pore. (Not all sections of taste buds will include the taste pore – why?) Taste hairs (microvilli) from the taste bud cells extend through the taste pores for chemoreception.

Compare the taste buds with interdigitations formed by the lamina propria and surface epithelium elsewhere in the slide.

Slides 43-44: Esophagus, Human & Rat (trichrome stain)

Scan the section at low power and observe the mucosa, submucosa, muscularis, and adventitia.

In the human, the mucosa is composed of a stratified squamous epithelium (with its component layers), and a thin lamina propria, and a muscularis mucosa. Deep to the muscularis mucosa is the submucosa, composed of loose connective tissue. Compare the muscularis in the two specimens to determine the level of the esophagus. Look between the muscle layers for large, basophilic cells. These are part of the myenteric plexus, postganglionic neurons of the parasympathetic nervous system. The adventitia is composed of loose connective tissue; look for blood vessels, nerves, and adipose tissue.

The rat tissue is stained with trichrome stain, which stains collagen green and nuclei red.

Slide 45: Cardioesophageal junction, Rat

At low power, find the esophageal end of the section. The mucosa of the esophagus consists of a stratified squamous keratinized epithelium, a thin lamina propria, and a muscularis mucosa. All three layers follow the undulations of the esophageal surface. Note that the rat esophageal epithelium is keratinized, while the human is not. The submucosa is mainly loose connective tissue. The muscularis consists of two layers of smooth muscle fibers: an inner circular, and an outer longitudinal layer. Notice that the fibers in one layer are cut in longitudinal section, while the fibers in the other are cut in cross section. If you see groups of 2-3 large, round to oval, basophilic nuclei in between the two muscle layers, they are neurons of the myenteric plexus of Auerbach, part of the autonomic nervous system. The thin lining deep to the muscle is the serosa.

Next, examine the stomach portion of the slide. At low power, notice the 3-4 folds of mucosa and submucosa; these are rugae. At higher power, you can see the surface is lined by a simple columnar epithelium made up of mucous surface cells. The apical area of the cells is lightly staining because the mucus secretory material has washed out, and there is a round/oval, basophilic nucleus at the base of the cell. The inner surface of the stomach is irregular because of numerous shallow depressions, the gastric pits, which are also lined by mucous surface cells. Opening into the base of the gastric pits are the gastric glands, which are long, tubular glands. While there are several types of cells in the gastric gland, it is practical to distinguish just three: mucous neck cells, which are located at the junction between the gastric pit and gland; parietal cells, which are rounded cells with a central spherical, basophilic nucleus, and an eosinophilic cytoplasm; and chief (zymogenic) cells, which have a basophilic cytoplasm and nucleus. The parietal cells secrete hydrochloric acid, and the chief cells secrete pepsinogen. In different parts of the section, the gastric pits are cut in both longitudinal and cross sections.

The gastric glands are located in the lamina propria, which otherwise is made up of loose connective tissue. Deep to the lamina propria is the muscularis mucosa, composed of a sheet of about 4-5 smooth muscle fibers thick. The submucosa is a layer of loose connective tissue between the mucosa and the muscularis. In this section, the muscularis appears to have two layers of smooth muscle fibers, but in the human there are usually three. Lining the deep surface of the muscularis is the serosa.

Slides 46-47: Stomach, Human & Rat

The same structures seen in the previous slide are present. In the lamina propria at the base of the gastric glands, there are areas with numerous eosinophil-like cells (rat).

Slide 49: Small Intestine, Rat.

Scan the slide at low power and find the cross section through the small intestine. There are no plicae circulares visible, but you can see the villi and intestinal glands at low power.

At higher power, study the surface epithelium composed of absorptive cells. They have oval nuclei in their base, and a brush border at their apical surface. Scattered among them are goblet cells.

Opening into the base of the villi are intestinal glands. Three of the cell types can be seen in the glands: absorptive cells, goblet cells, and, at the base of some of the glands, Paneth cells (these may be difficult to find/see), which have eosinophilic secretory granules in their apical cytoplasm. Try to find examples of mitotic figures deep in the intestinal glands, too.

The lamina propria is loose connective tissue which is highly infiltrated by different types of cells, and also contains blood vessels and a lacteal within each villus.

There is a very thin muscularis mucosa that may be hard to distinguish, and a thin submucosa.

The two layered muscularis has several neurons of the myenteric plexus visible.

Slide 48: Small intestine, Human

Look for examples of Paneth cells at the base of the intestinal glands.

Slide 51: Large Intestine, Rat.

Notice the structure of this organ at low power. In comparison to the small intestine there are no villi. There are a few infoldings of mucosa and submucosa; these are not present in the human.

The surface epithelium is a very uniform simple columnar epithelium of cells that absorb water. Opening onto the surface area are intestinal glands, which are lined by absorptive cells and goblet cells.

The lamina propria and submucosa are similar to the corresponding layers in the small intestine.

The organization of the muscularis is not precisely clear in this section, but in the human consists of an inner circular layer and a discontinuous longitudinal layer forming three bundles or strips (tenia coli)

Notice the fatty tissue associated with and part of the muscularis. This is an epiploic appendage.

Slide 52: Appendix, Human

The slide consists of a cross section of the vermiform appendix, which extends from the cecum and is therefore part of the large intestine. Notice the similarities with the large intestine slide. It characteristically has numerous lymph nodules and lymphocyte aggregations in the lamina propria and submucosa.

LAB 11: ACCESSORY ORGANS OF THE DIGESTIVE SYSTEM

Slide 53: Salivary gland, Human

At low power notice the lobules surrounded by adipose and loose connective tissue containing ducts lined by cuboidal to columnar epithelium, blood vessels, and nerves. The nuclei in all acini are clearly spherical, indicative of serous rather than mucous cells. In submandibular glands, serous cells predominate, in sublingual glands mucous predominates, parotid glands are all serous.

Slide 54: Salivary gland, Rat

The rat salivary gland is also divided into lobules, however, the connective tissue septa did not stain well, otherwise, this specimen has much better staining compared to the section of human tissue. The acini are stained with hematoxylin, and the ducts and connective tissue and blood vessels are stained with eosin.

The secretory cells in the acini have spherical nuclei and a light staining apical region, characteristic of serous cells.

The ducts are lined by a simple cuboidal/columnar epithelium and they coalesce to form larger and larger ducts which are located in the trabeculae. The acini empty into intercalated ducts, characterized by a small lumen. Intercalated ducts empty into striated ducts, characterized by a larger lumen and basal striations. Striated ducts in turn drain into interlobular ducts, with the largest lumen and located in the septa.

Slide 58: Pancreas,Human

At low power, observe the lobules of the pancreas formed by connective tissue septa, and drained by branches of the pancreatic duct. Among the densely staining acini of the exocrine glands, there are circular areas of light staining cells, the islets of Langerhans, which are the endocrine portion of the pancreas.

At higher power, note that the ducts are lined by simple cuboidal/columnar epithelium. This is in contrast to salivary glands, which have striated ducts. Acini are composed of serous cells which have a basally located nucleus and a basal cytoplasm which is typically basophilic. The apex of the acinar cells is eosinophilic, because of the presence of zymogen granules.

Slide 55: Liver, Human

Use low power to scan the section and note that three sides of the specimen were cut during dissection but that the fourth side has a covering of visceral peritoneum. Numerous blood vessels are visible; the larger ones are within connective tissue septa. Otherwise, the specimen is composed of liver tissue.

At medium power note that there are two kinds of blood vessel groups. In one, there is a single vein or several (~5-0) vessels. This is a central vein. In the other arrangement, there are one or more veins, arterioles, and hepatic ducts, which are lined by cuboidal epithelium. This group is a portal triad. Most of the central veins and portal triads are cut in cross section, but some are in oblique section.

Towards one corner there are a medium sized artery and vein, and a hepatic duct lined by columnar epithelium. These are branches of the hepatic artery, hepatic portal vein, and the hepatic bile duct.

Hepatocytes are cuboidal cells with an eosinophilic cytoplasm and a basophilic, spherical nucleus. The cells are arranged in sheets (cords) of one cell thickness. The cords of hepatocytes branch and form a network.

In general, cords of hepatocytes radiate from a central vein and form a functional unit, the liver lobule. In cross sections, liver lobules are hexagonal, with portal triads at the peripheral points between adjacent lobules. (See diagrams in text and atlas).

Blood flows from portal triads to central veins. Central veins form a tributary system, which leads to the hepatic vein.

Use the 40x objective to observe hepatocytes. Note the spaces between hepatic cords. Endothelial cells form leaky hepatic sinusoids; you can see their thin cytoplasm and flattened nuclei. Between the sinusoid and hepatocytes is another cavity, the space of tissue. Blood flowing through sinusoids readily leaks into the space of Disse and bathes the free margins of the hepatocytes. Besides endothelial cells and hepatocytes, you may see blood cells and/or macrophages, or Kupffer cells. Occasionally Kupffer cells can be identified by dark granules in their cytoplasm (residual bodies), and oval nucleus and irregularly shaped cytoplasm, and their location in the sinusoid or space of Disse.

Relate the structure of the liver lobule to the flow of blood and to the functions of the liver.

LAB 12: RESPIRATORY SYSTEM

Slide 62: Larynx, Rat

This section contains cross sections of both larynx and esophagus. The larynx is cut in the area of the laryngopharynx because it is lined by both stratified squamous keratinized and pseudostratified columnar (ciliated) epithelium. In the lamina propria are mixed seromucous glands, and the muscularis contains striated muscle fibers. Between the esophagus and laryngopharynx are mucous glands. Notice the cartilage which maintains the air passageway.

Slides 60, 76: Trachea, Human & Rat

Scan the tissue section at low power. Look for parts of the cartilaginous ring and the lining epithelium. The glandular tissue around the trachea are thyroid follicles.

The tracheal cartilage is hyaline and in the shape of a "C". The ends of the "C" are joined by smooth muscle fibers of the trachealis muscle. The cartilage is located in the lamina propria. Typical respiratory epithelium lines the trachea: pseudostratified columnar ciliated epithelium. In the lamina propria are mixed seromucous glands.

In the connective tissue around the thyroid are cross sections of myelinated nerves.

Slide 63-64: Lung, Human & Rat

In these slides, look at aveoli in the human tissue, and for respiratory passageways in the rat tissue. Many of the lung structures can been seen at low or medium power: alveoli, alveolar ducts, respiratory bronchioles, terminal bronchioles, bronchioles, blood vessels, and the visceral pleura.

In the alveolar wall, look for: type I alveolar cells, they will be squamous cells; type II cells, they will be cuboidal; capillaries; and macrophages, which will be cells with an irregular shape (pseudopodia) and located either in the alveolar lumen or in the alveolar wall. There are several other types of cells in the alveoli, but they are difficult to identify.

Alveolar ducts are passageways lined by alveoli.

Respiratory bronchioles can be identified by their darker staining edges, due to smooth muscle fibers, and the scattered alveoli opening into the lumen. The epithelium is squamous.

Terminal bronchioles do not have alveoli, and the epithelium may be simple cuboidal and/or simple squamous.

The largest bronchiole in the tissue section has simple columnar ciliated epithelium, which is a transition between the pseudostratified columnar in the primary bronchi and the simple squamous in the terminal bronchioles. There are also smooth muscle fibers in the lamina propria, and no cartilaginous plates.

NOTE: Lungs with few, enlarged alveoli may be pathological examples of emphysema where alveolar walls are damaged.

LAB 13: SKIN

Slide 66: Thick skin, Human, Slide 4: External ear

This sample was taken from the sole of the foot. Use low power to identify the epidermis, dermal papillae, ridges, the reticular layer of the dermis, and the subcutaneous layer. At higher power, find the stratum basale, stratum spinosum, stratum granulosum, and stratum corneum of the epidermis.

Examine the dermal papillae. They are composed of loose connective tissue with numerous capillaries. Present, but not visible in this slide, are Meissner's corpuscles (fine touch receptors).

The reticular layer of the dermis contains sweat ducts (stratified cuboidal epithelium), often surrounded by lymphocytes, blood vessels, and peripheral nerves, all surrounded by loose and dense irregular connective tissue.

In the underlying subcutaneous layer are adipose cells, eccrine sweat glands, blood vessels, peripheral nerves, and occasionally Paccinian corpuscles, which are pressure receptors. Paccinian corpuscles have the distinctive structure in sections of concentric layers of supporting cells, much line a horizontal section of an onion.

Review all of the different tissues on this slide.

Why do you think there are no/very few mitotic figures in the stratum basale?

Slide 42: Thin skin, Human; 4: external ear

The outer surface of the lip is an example of thin skin. Observe the epidermis, dermis, and hypodermis.

The epidermis has a stratum basale, stratum spinosum, stratum granulosum, and a stratum corneum. Dermal papillae are largely lacking. The two layers of the dermis are not distinguishable, and both layers appear to be composed of dense irregular connective tissue. Scattered hair follicles are present with associated sebaceous glands. There are sweat glands in the hypodermis.

Slide 67: Scalp, Human

The scalp is an example of thin skin, with a thin epidermis, few dermal papillae, and an underlying dermis and hypodermis. Hair follicles surround the hair root and extend from the epidermis to the hypodermis, where the hair bulb is located. In the dermis you should see sweat glands and sebaceous glands. There are also numerous arrector pili muscles.

LAB 14: URINARY SYSTEM

Slide 68: Kidney, Human

First examine the slide at low power and observe the areas of cortex and medulla. If there is an artery at the corticomedullary junction, what is its name?

In the cortex, examine the renal corpuscles and identify the parietal layer of Bowman's capsule, the urinary space, and the glomerulus, which is mainly composed of endothelial cells and podocytes, the visceral layer of Bowman's capsule. Be on the lookout for the urinary pole and the vascular pole of the renal corpuscle, but your section may not have them.

Most of the cross sections between renal corpuscles in the cortex are proximal convoluted tubules. The tall cuboidal cells have an eosinophilic cytoplasm and a basophilic nucleus, but the nuclei are relatively scattered. The apical surfaces of the cells make an irregularly shaped lumen; the cells have a brush border composed of microvilli.

Distal convoluted tubules are less numerous and can be distinguished because the cells are cuboidal and smaller than those in the proximal tubules. In addition, the cytoplasm is pale staining, and the basophilic nuclei are more numerous in cross sections, because of the smaller cell size. The lumen is proportionately larger because of cell size and the lack of a brush border.

In the medulla, there are mainly ascending and descending thick limbs of the loop of Henle, and blood vessels. The two limbs can be distinguished by their similarities to proximal and distal convoluted tubules. You should also try to observe the thin limbs of the loop of Henle and blood vessels. The collecting ducts travel toward the papilla and are characterized by pale cytoplasm, basal nuclei, and distinct intercellular boundaries. Be able to distinguish thick and thin limbs, collecting ducts, and blood vessels.

Slide 69: Kidney, Rat

The same structures seen in the human tissue should be found in the rat section. However, the proximal convoluted tubule epithelium appears to be low cuboidal, while the distal convoluted tubule epithelium seems to be the same height and similarly stained. Distal convoluted tubule epithelia have more nuclei per cross section, and lack a brush border. Notice the basal striations in the epithelial cells in both proximal and distal convoluted tubules; these are characteristic of cells that exocytose material. The brush border in the proximal convoluted tubule is also visible.

Other structures in the cortex are numerous blood vessels. You may be able to see a macula densa if you can find a distal convoluted tubule adjacent to a renal corpuscle and there is a concentration of epithelial cells (nuclei) in the tubule.

In the rat medulla, there are both thick and thin parts of the loop of Henle, but collecting ducts are difficult to see.

Slide 70: Ureter, Human

This is a cross section. Examine the transitional epithelium around the star shaped lumen. The surface cells are not squamous, but more oval, and the epithelium is clearly stratified. There is a mucosa, a submucosa, a muscularis (of smooth muscle fibers), and an adventitia. The orientation of the muscularis layers is inner longitudinal and outer circular.

Slide 71: Urinary bladder, Pig

At low power, note the layers of the bladder: mucosa, muscularis, and adventitia. The mucosa consists of transitional epithelium and a lamina propria. The transitional epithelium is clearly several nuclei in thickness, and the surface cells are larger than the others. The lamina propria consists of loose connective tissue with several blood vessels. Several separate bundles (rather than layers) of smooth muscle fibers make up the muscularis. In some areas, there is fatty tissue surrounding the muscularis.

LAB 15: ENDOCRINE SYSTEM

Slide 72: Pituitary, Rat

At low power, you should be able to discern three regions: pars distalis (the largest area), pars nervosa, and pars intermedia, located in between the two larger zones.

In pars distalis, there are unstained chromophobe cells, and acidophils and basophils (which may be difficult to see in this preparation), which make up the chromophil cells.

The pars nervosa is made up of unmyelinated nerve fibers; the oval nuclei belong to pituicytes, which are supporting cells.

Located between pars nervosa and pars distalis is pars intermedia. Pars intermedia and pars nervosa form the posterior lobe of the pituitary gland. The function of pars intermedia is not known.

Slide 73: Adrenal gland, Human

At low power, notice the capsule around the gland. Outside the capsule are different structures: fat tissue, blood vessels, and nerves (what type of nerves would you expect?). Within the capsule examine the cortex and the medulla. At medium and high power, examine the layers of the adrenal cortex. The layering is not always well organized, so try to find an area that looks like your atlas first.

The zona glomerulosa is located subjacent to the capsule. The cells have a round, basophilic nucleus, with a uniformly basophilic cytoplasm. Many of the cell clusters appear to have shrunken away from the stroma. Within the stroma are numerous capillaries, containing eosinophilic red blood cells.

At low/medium power the arrangement of cells in cords in the zona fasciculata can be seen, along with parallel blood vessels, also oriented perpendicularly to the capsule. With high power, the cells in the zona fasciculata can be seen to have a lighter staining nucleus and cytoplasm compared to the zona glomerulosa. The paleness of the cytoplasm is due to numerous lipid droplets (steroid hormone), which were washed out during processing.

The zona reticularis is located between zona fasciculata the adrenal medulla. The cells stain and look very much like the zona glomerulosa, except that some of them contain lipofuscin pigment (a pale brown appearing material) in their cytoplasm.

The adrenal medulla shares a very irregular border with the adrenal cortex. Medullary cells (chromaffin cells) typically have a light staining nucleus and a pale staining cytoplasm that often looks like the cytoplasm has shrunken away from the surrounding stroma. An occasional large nucleus may belong to a sympathetic ganglion neuron (difficult to find). Bundles of preganglionic axons are common.

Slide 74: Adrenal gland, Rat

With minor differences, the structure of the rat adrenal gland is very similar to that in the human. Try to find the same structures.

Slides 75,76: Thyroid gland, Rat

The thyroid gland is made up of follicles. The follicular cells secrete thyroid hormone which is stored as colloid in the lumen. Taller follicular cells are an indication of a more active cell.

Between thyroid follicles are loose connective tissue and parafollicular cells (these are hard to distinguish in normal light microscopy). The parafollicular cells secrete calcitonin, which acts to decrease blood calcium levels.

Slide 76: Parathyroid gland, Rat

The parathyroid glands are located next to or embedded within the thyroid glands. In this section, find the thyroid follicles and look for a mass of cells with a few connective tissue trabeculae; this is the parathyroid gland. Most (if not all) of the visible cells are chief cells, which secrete parathyroid hormone which increases blood calcium levels.

Slide 58: Pancreas, Human

Examine the pancreatic lobules at medium power. You should be able to find a few clusters of pale-staining cells among the pancreatic acini. Each cluster is an islet of Langerhans. While islets are comprised of four types of secretory cells, they are not distinguishable in an ordinary H & E preparation.

LAB 16: MALE REPRODUCTIVE SYSTEM

Slide 85: Testis, Rat

Low power (40X) The tunica albuginea forms a thin capsule that encloses the seminiferous tubules. There are no septa in the rat so the organ is not subdivided into lobules. In general , the connective tissue is the rat testis is not as well developed as in the human. The spaces between seminiferous tubules are artifacts induced when the section was floated in the tissue bath. Medium power (100X) Sections of the seminiferous tubules were cut at various orientations (cross, oblique, longitudinal) and occupy most of the testis. Small folds are common in the sections, which are 5 μm thick. Except for blood vessels, there is scant tissue between the seminiferous tubules. Notice also that the sample is almost exclusively seminiferous tubules, with one other tubule at the periphery. Missing in this slide are straight tubules, rete testis, efferent ducts, and epididymis.

(High power 400X) Between seminiferous tubules there are artefactual spaces with blood vessels and connective tissue. Leydig (interstitial) cells are not abundant, but scattered candidate cells may be seen with dense nuclei and a frothy, oval, eosinophilic cytoplasm.

Around the seminiferous tubules is a thin layer of fibroblasts and/or myoid cells.

In general, there are two types of cells next to the basal lamina of the seminiferous tubules: spermatogonia and Sertoli cells. Spermatogonia have round, basophilic nuclei. Sertoli cell nuclei have a prominent nucleolus, are irregular in shape, and are less densely stained. Unlike human Sertoli cells which have nuclei in the center of the epithelium, the rat Sertoli nuclei are at the basement membrane. They have a prominent nucleolus, with little heterochromatin.

In general, primary spermatocytes will be located next to spermatogonia, and secondary spermatocytes will be located next to primary spermatocytes. Their morphology does not provide any other way to differentiate them in this preparation.

Spermatids can be observed at different stages of spermiogenesis, which varies in different regions of the seminiferous tubules. Spermatids are most easily identified by their condensed nucleus. Prior to maturation, hook-shaped nuclei are located close to the basement membrane At this stage of spermiogenesis, you can see cellular debris in the lumen. This is the result of spermatids shedding excess cytoplasm, which will be phagocytized by the Sertoli cells. At more mature stages, highly condensed nuclei (sperm heads) are located at the apical surface of the seminiferous tubule, with sperm tails in the lumen.

Slide 86: Epididymis, Human and Rat

The epididymis is a single, coiled duct that is part of the duct system of the male reproductive tract where sperm mature and are stored. The epididymis is lined by pseudostratified columnar epithelium with stereocilia. The duct wall also contains a muscularis, capable of peristaltic contraction to move the sperm towards the urethra.

Slide 87: Ductus (vas) deferens, Human

This is a cross section of the spermatic cord, containing numerous blood vessels, nerves, and the ductus deferens. The ductus deferens is continuous between the epididymis and the ejaculatory duct, which enters the prostatic urethra. It is characterized by a narrow lumen lined by pseudostratified columnar epithelium with stereocilia (poorly fixed in this preparation) and a thick muscularis. You should be able to recognize an inner longitudinal, middle circular, and an outer longitudinal smooth muscle layer.

Slide 88: Prostate gland, Human

At low power, note the numerous tubuloacinar glands that make up the prostate. At high power, observe the pseudostratified columnar epithelium of the glands. Prostatic concretions are the eosinophilic structures in the lumen. Numerous nerves and blood vessels are located near the capsule.

Slide 89: Seminal vesicle, Human

The lumen contains secretory material, seminal fluid. Note the irregular surface created by complex folds and anastomoses of epithelium and lamina propria; this is characteristic of the seminal vesicle. While the epithelium in this gland is usually pseudostratified columnar, in this specimen it is simple columnar in many areas.

Deep to the lamina propria is a muscularis composed of several layers of smooth muscle fibers running in different directions. Around the muscularis is an adventitia of loose connective tissue with nerves, arteries, and veins..

Slide 90: Corpus spongiosum, Human

The penis contains three cylindrical masses of erectile tissue: two corpora cavernosa, and the corpus spongiosum. This is a cross section of the corpus spongiosum. Around the periphery is a layer of dense irregular connective tissue, the tunica albuginea. The corpus spongiosum consists of venous spaces making up the erectile tissue; separating the venous spaces are connective tissue

trabeculae containing blood vessels, smooth muscle, and nerves. The cavernous urethra runs through the corpus spongiosum and is lined by pseudostratified columnar epithelium. There are outpocketings of the cavernous urethra which may be continuous with the urethral glands. Superficial to the tunica albuginea are several veins which form the means for drainage of the venous spaces.

LAB 17: FEMALE REPRODUCTIVE SYSTEM & SENSORY RECEPTORS

Slides 77, 81: Ovary (and oviduct), Cat & Rat

Note: look for developing follicles in the cat slide; corpora lutea in the rat slide.

At low power, notice the ovarian follicles in the cortex around a loosely organized medulla. Peripheral to the ovary are sections through the oviduct. At high power, examine the surface of the rat ovary covered by a simple cuboidal "germinal" epithelium, which was mistakenly named by early histologists.

In the cortex, look for different stages of follicular development: primordial follicles, which are primary oocytes with a simple squamous layer of follicle cells; primary follicles, oocytes with one or more layers of cuboidal follicular cells; secondary follicles, a follicle with an antrum and a cumulus oophorus; and mature follicles, with an enlarged antrum and a corona radiata. In most instances, you will actually see only partial follicles, because of the orientation of the section. There will also be atretic follicles (which may contain lipofuscin granules) because follicle development can degenerate at any stage, and there may also be several corpus lutea, especially in rat tissue. In addition, look for a zona pellucida surrounding primary oocytes.

In a primary, secondary, or mature follicle, examine the cells surrounding the follicular cells and located outside of the basal lamina. The first layer is composed of theca interna cells, while the outer layer is made up of theca externa cells which imperceptibly merge with the surrounding connective tissue. Many of the blood vessels contain red blood cells because the organ was fixed by immersion.

Among the cells between follicles, there are clusters of large interstitial cells. They are rich in lipids and secrete estrogen. The cells originate from theca interna cells that persists and enlarge from atretic follicles. These interstitial cells are less well developed in human ovaries.

Adjacent to the ovary, notice the fimbriae of the distal end of the oviduct. The epithelium is composed of simple columnar cells some of which are ciliated while others are secretory. In the muscularis look for the inner circular and outer longitudinal bundles of fibers. This area of the oviduct is the fimbria. Look for lymphatics in this area.

Slides 80, 82: Uterus, Cat

The oviduct is a tube like structure which transports ova from the ovary to the uterus. The wall of the oviduct has three layers: a mucosa, a muscularis, and a serosa. The mucosa varies along the length of the oviduct. It is most complex at the distal end, or fimbria, where numerous branched folds form finger like projections which greatly increase the surface area. Towards the uterus the epithelial folds become less and less complex.

The epithelium is simple columnar with secretory and ciliated cells. The secretory cells produce a liquid covering that nourishes sperm and allows them to mature (capacitation). The cilia beat towards the uterus, to help transport the ova.

The outer layers of the oviduct are a thin lamina propria, a muscularis, and an outer serosa, which is not well preserved in this specimen. In the cat, there are glands in the lamina propria which are not present in the human.

Slide 78: Uterus, Pig

This is a sample of uterine wall from the body of the uterus. The three main layers are the endometrium, the myometrium, and the serosa.

The surface epithelium of the endometrium is not well preserved, but you can note its irregular surface and the simple columnar epithelium. There are numerous lymphocytes among the epithelial cells and in the lamina propria.

The lamina propria is loose connective tissue with numerous uterine glands, arteries, and veins. The thickness of the lamina propria varies with the phases of the menstrual cycle. This specimen is in the proliferative phase, when all components in the lamina propria are growing (see text Chapter 23). The uterine glands are simple tubular glands, lined by simple columnar epithelium. The blood vessels also proliferate. At menstruation, the superficial portion of the uterine wall (functionalis) is sloughed off including the coiled arteries, leaving only the deeper basalis and straight arteries. Components of the basalis then proliferate to form a new functionalis.

In this specimen the myometrium is composed of two layers of smooth muscle fibers, arranged perpendicularly. In human, the muscle fibers do not form such distinct layers.

Lining the myometrium is a simple cuboidal epithelium which is part of the serosa.

Slide 84: Placenta, Human

The placenta consists of many islands of fetal tissue of varying sizes (chorionic villi which in life were bathed by maternal blood in the empty spaces of this tissue section. Examine several chorionic villi at high power; the regular, single row of oval nuclei lining the villus surface is the syncytiotrophoblast. The cytotrophoblast deep to the syncytiotrophoblast is scattered and difficult to distinguish from the lamina propria (it disappears during the later stages of pregnancy). Notice the numerous fetal blood vessels in the lamina propria.

LAB 18: EYE

Slide 93: Eye, Human

For most sections, the eye is cut through the anterior-posterior axis, including the optic papilla. Examine the section without the microscope initially, to become oriented, then begin with low power. In most sections, the brittle lens has cracked during sectioning, and is partially missing. In addition, the handling and processing caused an artifactual detachment of the retina from the retinal pigment epithelium, and a detachment of the retinal pigment epithelium and part of the choroid by splitting of the choroid layer.

In the cornea, look for the five constituent layers: the corneal epithelium, composed of stratified squamous epithelium; Bowman's membrane (basal lamina); the stroma, which makes up the bulk of the cornea and is composed of collagen; Descemet's membrane (basal lamina); and the corneal endothelium, a simple squamous/cuboidal epithelium.

The sclera is a tough, protective shell for the eye. It is composed of dense irregular connective tissue, which is covered by conjunctiva anteriorly.

At the inner surface of the corneoscleral junction, anterior to the iris, is a fenestrated lining called the trabecular meshwork. Crevices of the meshwork lead to a circular vessel called the canal of Schlemm. The canal of Schlemm is only partially intact on our specimens. The area between the iris and the cornea is called the anterior chamber. In life it is filled with aqueous humor which drains out of the anterior chamber via the trabecular meshwork and canal of Schlemm, which lead to veins outside of the eye.

The choroid is the middle layer of the eye between the retina and the sclera. It is composed of loose connective tissue with numerous blood vessels and melanocytes. Between the choroid and the pigment epithelium is a glassy membrane (basal lamina).

Anteriorly, the choroid layer thickens to form the ciliary body. Within the ciliary body is the ciliary muscle. On the surface, the ciliary body is irregular, due to the ciliary processes. Lining the surface is the ciliary epithelium, which has a layer of pigmented cells covered by a layer of unpigmented cells. The ciliary epithelium continuously secretes aqueous humor into the space between the lens and the iris, the posterior chamber. The suspensory ligaments are fibers running between the ciliary processes and the lens, holding the lens in place and controlling its shape, for focusing. The fibers do not stain well and are therefore difficult to see.

The iris is anterior to the lens and forms an opening, the pupil. The iris is composed of loose connective tissue, with a heavy concentration of pigment on its posterior surface, to prevent the passage of light. The size of the pupil is controlled by two sets of smooth muscle fibers: the dilator muscle, with radially arranged fibers; and the sphincter muscle, with circularly arranged fibers.

The retina forms the inner layer of the eye. It extends from the ora serrata posteriorly to the optic papilla, and continues around to the ora serrata on the opposite side. The two main parts of the retina are the retinal pigment epithelium and the neural retina.

The retinal pigment epithelium is a simple cuboidal epithelium located between the neural retina and the choroid. The retinal pigment epithelium can be identified by the presence of melanin, but in many parts of our specimen it is destroyed. A good place to look for it is near the optic papilla.

The neural retina is a highly organized structure with about seven layers easily seen. The outermost layer, next to the retinal pigment epithelium, is composed of the outer and inner segments of the photoreceptor cells. this part of the photoreceptor cells contains visual pigments which are light sensitive. There are two types of photoreceptors, rods and cones, based on the shape of their outer segments.

The second layer of the retina (the outer nuclear layer) is made up of the cell bodies of the photoreceptor cells, and is marked by their basophilic nuclei.

In the third layer (the outer plexiform layer), processes from the photoreceptor cells synapse onto interneurons.

The fourth layer (the inner nuclear layer) is composed of the cell bodies of several types of interneurons, and is seen as a layer of nuclei.

The fifth layer (the inner plexiform layer) contains synapses between processes of interneurons and retinal ganglion cells.

The sixth layer (the retinal ganglion cell layer) is made up of the cell bodies of retinal ganglion cells, and is the innermost layer of nuclei.

The seventh layer (the nerve fiber layer) is composed of axons from the retinal ganglion cells which are on their way towards the optic papilla. These axons convey retinal impulses to the brain via the optic nerve.

At the posterior pole of the eye, the axons of the nerve fiber layer exit the eye, forming the optic papilla. The axons must traverse the retina, choroid, and sclera (where they form the lamina cribosa). This interruption of the layer of photoreceptors creates the blind spot. The axons become myelinated upon leaving the eye, and form the optic nerve, which, being part of the brain, is covered by the three layers of the meninges: pia mater, arachnoid (and subarachnoid space with cerebrospinal fluid), and dura mater.

The lens is a crystalline-like structure derived from cells. Around the periphery of the lens is an extracellular lens capsule. Deep to the capsule on the anterior surface is a subcapsular epithelium. At the equator of the lens, cells of the subcapsular epithelium differentiate into the crystalline elements that make up the lens.

In the center of the eye, between the lens and the retina, is a clear, gel-like substance, the vitreous humor.

Slide 95: Optic papilla, Human

Use low power to orient yourself to the retina, sclera, and optic nerve. In the retina, notice the increasing thickness of the optic fiber layer as it approaches the optic papilla. The axons accumulate at the papilla from all areas of the retina. Note also the abrupt interruption of the layers of the retina as the optic axons make a 90° turn to exit from the eye. Along the optic nerve you may be able to see the beginning of myelination, and also the wrapping of pia, arachnoid, and dura, and the subarachnoid space.

Large vessels at the optic papilla are the central retinal artery and vein, which enter and leave the retina by traveling through the optic nerve.

LAB 19: EAR

Slide 98: Inner ear, Rat, Decalcified

Scan your section at low power. Samples vary so some slides will contain structures not found on others. You may find areas of external ear, with the external auditory meatus, lined by skin and supported by hyaline cartilage and temporal bone, and ending at the tympanic membrane. Note the sebaceous-like glands, responsible for ear wax.

Deep to the tympanic membrane is the middle ear cavity, lined by a simple squamous epithelium, and containing irregularly shaped bones, the middle ear ossicles (malleus, incus, and stapes). Look for the malleus attached to the tympanic membrane.

Deep to the middle ear cavity is the inner ear. Follow the epithelial lining of the inner ear to find areas of specialization: examples of bony and membranous labyrinth.. Your section should have an Organ of Corti, and may have a macula, and/or a crista ampullaris. Identify the hair cells and supporting cells; text diagrams will be helpful. In the area of the Organ of Corti, you should see bundles of nerve fibers and the spiral ganglion. Also, look for the vestibular membrane separating the scala vestibuli from the scala media, and identify the scala tympani.

Structures to look for in the Organ of Corti: inner and outer hair cells, tectorial membrane, basilar membrane, internal spiral tunnel, internal tunnel, supporting cells, stria vascularis.

Structures to look for in maculae: otoliths, gelatinous mass, hair cells.

Structures to look for in crista ampullaris: hair cells, cupula, supporting cells.

Additional structures/tissues to look for are: sensory ganglia and nerves; skeletal muscle fibers; mucous glands; bone; cartilage; osteoclasts; and blood vessels.