LABORATORY 18

URINARY SYSTEM

OBJECTIVES:

At the end of this lab, you should be able to describe, recognize, and/or identify:

1. the light microscopic structure of the kidney including: cortex, medulla, renal corpuscles, parts of the nephron, collecting ducts, vasculature, calyces, pelvis, and renal sinus

2. the structure of renal lobules vs. renal lobes

3. the electron microscopic appearance of the filtration barrier between the lumen of the glomerular capillary and the urinary space of the renal corpuscle

4. the electron microscopic appearance of the cells of proximal tubules, distal tubules, thin limbs of the loop of Henle, and collecting ducts

5. the light and electron microscopic features of the juxtaglomerular apparatus, and its function

6. the light microscopic features of the ureter, urinary bladder, and urethra

7. the location and characteristics of transitional epithelium

LABORATORY:

Please study the following slides in your set:

I. KIDNEY

At the microscopic level, the urinary system includes many named structures. Some of the most important are summarized here: Each kidney is composed of millions of nephrons. A nephron consists of multiple components connected in the following order:

- renal corpuscle
- proximal convoluted tubule
- straight portion of proximal tubule (= descending thick limb of the loop of Henle)
- thin limb of the loop of Henle
- straight portion of distal tubule (= ascending thick limb of the loop of Henle)
- distal convoluted tubule

The distal convoluted tubule is continuous with the collecting tubule, which empties into a collecting duct. The nephron plus the collecting duct form a uriniferous tubule. The collecting ducts open into a minor calyx at the tip of a renal pyramid. Minor calyces unite to form major calyces, which unite to form the pelvis of the ureter.
A. Renal Corpuscles:

Slide 73 (HU Box): Kidney, Whole Coronal Section, and Slide 50, 51 and 51A: Kidney

The kidney can be divided into a cortex and medulla. The cortex is found at the surface of the kidney in contact with the capsule and between the renal pyramids. It can be distinguished easily because of the presence of renal corpuscles. A renal corpuscle is composed of a glomerulus (a tuft of capillaries) and glomerular capsule (Bowman's capsule). Bowman's capsule has a visceral and a parietal layer. The visceral layer is composed of podocytes, which are best seen with the electron microscope. They cover the outer surface of the glomerular capillaries. The parietal layer is a simple squamous epithelium that lines the wall of Bowman's capsule. The visceral and parietal layers are continuous at the vascular pole of the renal corpuscle, which is also where the afferent and efferent glomerular arterioles enter and leave. The space between the visceral and parietal layers is the urinary space (Bowman's space), which receives the glomerular filtrate. At the urinary pole of the renal corpuscle the urinary space is continuous with the lumen of the proximal convoluted tubule, allowing the filtrate to enter the tubules of the nephron. At the urinary pole there is an abrupt change from the simple squamous epithelium of Bowman's capsule to the simple cuboidal epithelium of the proximal convoluted tubule.

The renal corpuscles located near the boundary between cortex and medulla belong to juxtamedullary nephrons. Although you cannot appreciate it in sectioned material, juxtamedullary nephrons have extremely long loops of Henle extending deep into the medulla. The renal corpuscles located further from the medulla belong to cortical nephrons and tend to have shorter loops of Henle.

B. Tubule Types

The cells of a proximal convoluted tubule look very much like those in the straight part of the proximal tubule, and the cells of the distal convoluted tubule look very much like those in the straight part of the distal tubule. You will see shortly that it is possible to distinguish between the straight and convoluted part of a tubule based on the location of the tubule within the kidney, but for the moment do not worry about whether you are looking at the convoluted or straight part of a tubule. Concentrate on comparing and contrasting the cells found in proximal tubules, distal tubules and collecting tubules.

The proximal tubules and distal tubules are both lined by a simple cuboidal epithelium. By LM they have faint basal striations, and the lateral boundaries between cells are indistinct. Why are the lateral plasma membranes not clearly visible on proximal or distal tubules by LM? (Answer: Because of extensive interdigitation of the lateral plasma membranes of neighboring cells. This interdigitation increases the surface area available for membrane transport.) At the EM level what cellular structures are responsible for the basal striations? (Answer: The striations are created by long rows of mitochondria arranged between the infoldings of the basal plasma membrane. The mitochondria provide the ATP to drive the active transport systems.) Proximal tubules can be distinguished from distal tubules because:

1. Proximal tubule cells have tall, closely packed microvilli on their apical surfaces forming a well-developed brush border. Often the brush border is not well preserved and partially sloughs off into the lumen, so that proximal tubules appear to have debris in their lumen. The cells of distal tubules have sparse microvilli, which are usually not evident except by electron microscopy.
2. Proximal tubule cells are often more eosinophilic than distal tubules.

3. Proximal tubules are made up of larger (wider) cells, and therefore fewer nuclei are present in an average cross section of a proximal tubule vs. a distal.

4. Proximal tubules are often wider in diameter than distal tubules.

5. The nuclei of distal tubules are located very close to the apical plasma membrane, sometimes seeming to bulge into the lumen. In contrast, the brush border of the proximal cells separates the nuclei of proximal tubule cells from the lumen and makes them appear more centrally or basally located in the cell.

Collecting tubules are lined by a simple cuboidal epithelium that lacks a brush border. The cytoplasm is usually quite pale. They can be distinguished from proximal and distal tubules because they have relatively obvious lateral boundaries between cells. As the collecting system passes deeper into the medulla, the epithelium gradually changes into a simple columnar epithelium. There are several different conventions for defining a collecting tubule vs. a collecting duct. Some books call the structure a tubule if it is in the cortex and a duct if it is in the medulla. Some call it a tubule if it has a cuboidal epithelium and a duct if it has columnar epithelium, regardless of tubule location. For our purposes, you should consider the structure to be a collecting duct if the epithelium is columnar or if the tubule is clearly located in the medulla.

The thin limbs of the loops of Henle are located in the medulla. They consist of a simple squamous epithelium just slightly thicker (taller) than capillary endothelial cells (i.e., intermediate in height between capillary endothelium and a distal tubule).

C. Subdivisions of the Cortex

The cortex can be subdivided into cortical labyrinths and medullary rays (yes, sorry, but medullary rays are part of the cortex.) A medullary ray contains the straight portions of proximal tubules, distal tubules and collecting tubules running parallel to one another to form a bundle of straight tubules. Medullary rays are oriented radially, running from the cortico-medullary boundary at the base of each pyramid toward the capsule, much like rays of light emanating from the medulla.

Cortical labyrinths contain renal corpuscles and the convoluted portions of the proximal and distal tubules. Cortical labyrinths are thus characterized by tubules running in many different directions instead of in a single direction as in the medullary rays.

Cortical labyrinths and medullary rays alternate with one another to produce a pattern of alternating stripes (the medullary rays) and more disorganized regions (the cortical labyrinths) in a coronal section of kidney. Compare areas where the medullary rays have been cut in longitudinal section to areas where they have been cut in cross section. Appreciate the fact that the medullary rays look very different in these two orientations, while the cortical labyrinths look the same in each because of the random orientation of the tubules they contain.

D. Medulla

The renal medulla consists of conical structures called pyramids. They are separated from one another by areas of cortex called the renal columns (columns of Bertin). Note that the base of each pyramid is oriented toward the surface of the kidney, and the apex toward the hilus. The apex of a renal pyramid is called the renal papilla. It has on its surface the openings of the large collecting ducts (ducts of Bellini). By SEM
these numerous openings give the area a sieve-like appearance, hence it is called the area cribrosa (Latin, cribrosa = sieve). The collecting ducts from one pyramid all empty into the same minor calyx at the area cribrosa.

The medulla is sometimes divided into an inner zone that contains only collecting ducts and thin limbs of Henle’s loop, and an outer zone that contains those tubule types plus straight parts of proximal and distal tubules. This difference in tubule types may be visible by LM as a darker coloration of the outer portion of the medulla. Notice that within a renal pyramid the various tubules run parallel to one another toward the renal papilla. The medulla does not contain convoluted tubules as in the cortical labyrinths.

E. Blood Supply, Lobes & Lobules

A type of blood vessel known as an interlobar artery runs near the center of each renal column, oriented perpendicular to the kidney surface. Interlobar arteries mark the boundaries between kidney lobes. A lobe is conical in shape. It includes cortex and medulla, and consists of one renal pyramid, the cortex between the base of the pyramid and the kidney capsule, plus roughly half of the renal column flanking it on each side. Functionally a lobe can be described as all the uniferous tubules that drain into the same minor calyx. Human kidneys are multilobar, i.e., contain more than one lobe. In the newborn the kidney surface is often indented between lobes, making them quite obvious. In the mature kidney these surface indentations disappear. Note that some versions of Slide 73HU must be from non-human material because the kidney on those slides is unilobar.

Interlobar arteries give rise to arcuate arteries, which run along the base of the pyramids parallel to the surface of the kidney. They give off interlobular arteries, which run approximately through the center of a cortical labyrinth, oriented perpendicular to the surface of the kidney. Interlobular arteries mark the boundary between kidney lobules. A lobule includes cortex but no medulla. It is cylindrical in shape and is centered on a medullary ray. A lobule includes the medullary ray plus roughly half of the cortical labyrinth flanking it on each side. Functionally a lobule can be described as all the nephrons that drain into the same medullary ray.

Although it is not necessary to distinguish between afferent and efferent glomerular arterioles in sectioned material, you should be aware that interlobular arteries give off multiple afferent arterioles which break up into glomerular capillaries, and that the glomerular capillaries merge to form the efferent arteriole that leaves each renal corpuscle. Efferent glomerular arterioles then break up into another capillary bed. Those from juxtamedullary nephrons form the vasa recta supplying the medulla, while those of cortical nephrons form the peritubular capillaries supplying the cortex. Realize, therefore, that the kidney contains an arteriolar portal system since the blood travels through two capillary beds (glomerular capillaries and then either vasa recta or peritubular capillaries) connected in series by an arteriole (the efferent glomerular arteriole). Contrast this with the venous portal system of the gut and liver. In the kidney the two capillary beds have different functions. The glomerular capillary bed filters the blood. Peritubular capillaries and vasa recta are more conventional in that they are concerned with supplying oxygen and nutrients to the cells of cortex or medulla, respectively. Peritubular capillaries drain into interlobular veins, which drain to arcuate veins and then interlobar veins, following the arteries of the same name. Vasa recta often drain directly into arcuate veins. Histologically, peritubular capillaries are distinguished from vasa recta by their location: peritubular capillaries are in the cortex and vasa recta in the medulla.
F. Juxtaglomerular Apparatus

Now locate a renal corpuscle that has been sectioned through its vascular pole. Look for a macula densa. It is a part of the distal convoluted tubule that arose from that same renal corpuscle. The cells making up the macula densa are narrower and taller than the other cells of the distal tubule, and therefore their nuclei will appear to be crowded closer together to produce a darker looking area on that side of the tubule (Latin, macula densa = dense spot).

The arteriole adjacent to the macula densa contains specialized cells called juxtaglomerular cells (sometimes called granular cells by physiologists) in its tunica media. They are modified smooth muscle cells and they secrete renin. The juxtaglomerular cells are most often found in the afferent arteriole, but occasionally in the efferent arteriole as well. Together with the macula densa and the extraglomerular mesangial cells, these structures comprise the juxtaglomerular apparatus. The extraglomerular mesangial cells are located in the roughly triangular area between the afferent arteriole, efferent arteriole and macula densa (Ross, Fig. 20.7, p.705 & Wheater, Fig. 16.18, p.308).

Slide 51B: Kidney PAS

This section has been stained by the periodic acid-Schiff (PAS) procedure, which stains carbohydrate-rich structures magenta. In the kidney it stains several structures including the glycocalyx, which covers the microvilli of the proximal tubules, making it easy to distinguish them from distal tubules whose microvilli are too sparse to produce visible staining. Also stained are the basement membranes of all types of kidney tubules and the unusually thick basement membrane of the glomerular capillaries.

II. LOWER URINARY TRACT

Slide 73 (HU Box): Kidney, Whole Coronal Section, and Slide 50, 51 and 51A: Kidney

Return to these previously studied slides to study the minor calyces, major calyces and renal pelvis. What type of epithelium lines the lumen of these structures? (Answer: Transitional epithelium, which is unique to the urinary system) Examine the fat-filled space surrounding the calyces and renal pelvis known as the renal sinus. It also contains the large branches of the renal artery and renal vein. Appreciate the relationship between the renal sinus and the hilus of the kidney (see Wheater, Figs. 16.2 & 16.3, p. 293). The hilus (or hilum) is the indentation on the medial side of each kidney where vessels enter and leave and where the ureter leaves the kidney by passing through a narrow slit. As you pass through that slit you enter the renal sinus. Therefore you can think of the renal sinus as being analogous to a cave, where the kidney tissue makes up the wall of the cave and the hilus is the cave entrance.

Note that the renal pelvis narrows at the hilus and becomes the ureter.

Slide 76 (HU Box): Ureter, Human, c.s., or Slide 53: Ureter

The ureter has a mucosa, a muscularis and an adventitia. There is no muscularis mucosae and therefore no submucosa in the ureters. The mucosa is lined with transitional epithelium, and is typically folded to produce an irregular lumen due to
contraction of the smooth muscle in the muscularis. The smooth muscle of the upper portion of the ureter is normally arranged in an inner longitudinal and an outer circular layer. Notice that this is the opposite of most parts of the GI tract where the muscularis has an inner circular and outer longitudinal layer. Near the bladder the ureter has an additional outer longitudinal layer, creating inner and outer longitudinal layers and a middle circular layer. Examine your slides to see if you can decide which arrangement is present. On many of our slides distinct muscle layers are unusually difficult to demonstrate.

Slide 82 (HU Box): Transitional Epithel., (Urinary Bladder-Contracted)
Slide 75 (HU Box): Urinary Bladder, Distended, c.s., and
Slide 54A, 54B or 54C: Urinary Bladder

The urinary bladder has a mucosa, a muscularis, and either an adventitia or serosa depending on which part of the bladder you are studying. The muscularis mucosae is extremely variable. In some individuals it is not present at all, while in others it may be discontinuous or a sparse continuous layer. It is sometimes difficult to distinguish from ureter, but normally the bladder has:
- a much wider lumen than the ureter
- a muscularis with smooth muscle that is more irregularly arranged than in the ureter
- more collagen between smooth muscle bundles in the muscularis of the ureter
- a relatively thicker mucosal layer

Slide 54C includes the mesothelium, which lines the peritoneal cavity. This mesothelium contacts the superior surface of the bladder to create a serosa on that surface. The remaining parts of the bladder have an adventitia.

Slide 84 (HU Box): Penis, Fetal, Masson Trichrome, or
Slide 85: Penis
and
Slide 91 (HU Box): Urethra, Female, c.s., or
Slide 94 and 94A: Urethra and Vagina

Compare the male and female urethras. The male urethra has three parts: prostatic (as it passes through the prostate gland), membranous (as it passes through the skeletal muscle of the urogenital diaphragm), and penile (as it passes through the corpus spongiosum of the penis). The penile urethra is seen on these slides. Identify the urethra, which is surrounded by the erectile tissue of the corpus spongiosum. Be aware that the epithelium of the male urethra changes along its length. The first part of the prostatic urethra is lined by transitional epithelium, which then changes to pseudostratified columnar with patches of stratified columnar, and finally to stratified squamous as it approaches the external orifice of the penile urethra.

The female urethra is much shorter but the epithelium undergoes the same changes along its length. Observe the arrangement of smooth and skeletal muscle surrounding the female urethra in slide 94. The smooth muscle stains a pale grayish purple color and is located mainly internal to the skeletal muscle. The skeletal muscle is the voluntary sphincter of the urethra.
III. ELECTRON MICROSCOPY

A. Renal Corpuscles & the Glomerular Filtration Barrier

The scanning electron microscope helps to elucidate the relationship of the podocytes to the glomerulus (Ross, Fig. 20.12, p. 709). Make sure you can identify: podocytes, and their primary processes, secondary processes, and pedicels (foot processes). In the TEMs in Rhodin identify:

- podocytes (Figs. 32-9 & 32-10) and their pedicels (Fig. 32-10)
- glomerular endothelial cells (Figs. 32-9 & 32-10)
- extraglomerular mesangial cells (near #10 in Fig. 32-9)
- intraglomerular mesangial cells (#9 in 32-9, #6 in 32-10). They are located between the endothelial cells and the basal lamina.
- the parietal layer of Bowman’s capsule (Figs. 32-3 & 32-9)
- the urinary space vs. the lumen of glomerular capillaries (Figs. 32-3, 32-9 & 32-10)

In (Fig. 32-12) note that at the urinary pole of the renal corpuscle there is an abrupt change in the height of the epithelium from simple squamous in the parietal layer of Bowman’s capsule to simple cuboidal in the proximal convoluted tubule.

Note that at the vascular pole of the renal corpuscle (Fig. 32-9) the visceral and parietal layers of Bowman’s capsule are continuous with one another. Nearby are the macula densa, afferent arteriole, and efferent arteriole.

On the left side of Fig. 32-11, study the minimum filtration barrier (#3) between glomerular capillary lumen (#1) and urinary space (#2). What are the layers of this barrier? (Answer: From capillary lumen to urinary space the layers are capillary endothelium, fused basal laminae of endothelial cells and podocytes, and the filtration slit membrane between pedicels of the podocyte. Note that the fused basal laminae are commonly referred to as the “basement membrane” even though there is no lamina reticularis.) What is unusual about the endothelial cells of the glomerulus? (Answer: The fenestrations in the endothelial cell cytoplasm have no diaphragms)

B. Types Of Renal Tubules

Identify proximal tubules (Figs. 32-3, 32-13 & 32-14). Observe the well-developed brush border, the extensive infoldings of the basal plasma membrane, the numerous mitochondria lined up in those folds, and the junctional complexes between cells. Also note the numerous endocytic vesicles and lysosomes in the apical half of the cells. These are involved in the reabsorption and digestion of proteins from the glomerular filtrate.

Compare the proximal tubules with distal tubules (Figs. 32-3, 32-19 and 32-20). Note the sparse microvilli, the shorter distances between nuclei (due to the smaller size of distal tubule cells) and the bulging of some of the nuclei into the lumen of the distal tubule. Distal tubules still have a considerable number of mitochondria because they are also involved in ion transport.

Compare proximal and distal tubules with collecting ducts (Fig. 32-23). Observe that whereas it is very difficult to find the lateral intercellular borders in proximal or distal tubules, they are quite easily visible in collecting ducts.

Identify the thin limbs of the loop of Henle (Fig. 32-18 & 32-28) and compare them with the vasa recta. The epithelial cells of the thin limb are usually not quite as squamous as the endothelial cells of the vasa recta.
Realize that the capillaries seen in Figs. 32-3 & 32-9 are properly called peritubular capillaries rather than vasa recta. Why? (Answer: Any capillaries located in the cortex are referred to as peritubular capillaries. They form an irregular network that surrounds and supplies individual kidney tubules. Capillaries in the medulla are referred to as vasa recta. They form bundles of straight vessels that run parallel to the loops of Henle.)

C. Transitional Epithelium

Review the appearance of transitional epithelium (Figs. 32-33 through 32-35). Note the numerous flat fusiform vesicles in the cytoplasm of the apical cells (labeled “flat discoid vesicles” in Fig. 32-35). These are apparently invaginations of the plasma membrane rather than true vesicles. What is their presumed function? [Answer: They can apparently unfold rapidly (like opening a book) to increase the surface area of the epithelial cell plasma membrane as the bladder distends; when the bladder empties, these vesicles refold and are held in reserve until needed again.]
LABORATORY 18 CHECKLIST
URINARY SYSTEM

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<td>cortex of kidney</td>
<td>renal pyramid</td>
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<td>medulla of kidney</td>
<td>renal papilla</td>
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<tr>
<td>renal corpuscle</td>
<td>renal column (of Bertin)</td>
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<td>glomerular (Bowman’s) capsule</td>
<td>area cribrosa</td>
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<td>urinary space</td>
<td>minor calyx</td>
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<td>podocyte</td>
<td>major calyx</td>
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<td>interlobar artery</td>
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<td>macula densa</td>
<td>arcuate artery</td>
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<td>juxtaglomerular cells</td>
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<td>glomerular capillaries</td>
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<td>vasa recta</td>
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<td>renal sinus vs. hilus of kidney</td>
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<td>distal convoluted tubule</td>
<td>ureter</td>
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<td>collecting tubule/duct</td>
<td>mucosa, muscularis &amp; adventitia/serosa of ureter &amp; urinary bladder</td>
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<td>duct of Bellini</td>
<td>upper vs. lower end of ureter</td>
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<td>renal lobe vs. renal lobule</td>
<td>urinary bladder (serosa vs. adventitia)</td>
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<td>juxtamedullary nephron</td>
<td>penile urethra</td>
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<th>ELECTRON MICROGRAPHS</th>
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<td>Transitional epithelium</td>
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**NOTE:** These checklists include MOST of the structures that you should be able to identify. Exams may include structures not on these lists.
FOCUS QUESTIONS
LAB 18: URINARY SYSTEM

See whether you can answer the following questions. The correct answers are posted on the course website (http://neurobio.drexelmed.edu/education/ifm/microanatomy) under “Lab Focus Questions”.

1. Why do proximal tubules have numerous lysosomes?
2. What is the distinction between a kidney lobe and lobule?
3. The medulla of the kidney can be divided into inner medulla vs. outer medulla, and the outer medulla can be subdivided into an inner stripe and outer stripe. The boundaries between these regions are created by the different distances to which different tubule types penetrate into the medulla. What creates the division between inner medulla and outer medulla? Between inner and outer stripes of the outer medulla?
4. A portal system exists in the kidney. Is it an arterial or venous portal system? Where are the two capillary beds located?
5. Why is it important for the vasa recta to loop down into the medulla and then back up toward the cortex, following the loops of Henle fairly closely? What would happen if they ran straight down from the cortex, through the medulla, and exited at the renal papillae rather than forming a loop?
6. Describe the juxtaglomerular cells. Where are they located, what is their main product?
7. What stimuli cause the JG cells to secrete their hormonal product?
8. How does renin affect kidney function?
9. Describe the structure, location and function of podocytes.
10. Describe the structure, location and function of intraglomerular mesangial cells.
11. What are the three components of the minimal glomerular filtration barrier? Which is usually the most important for regulating the passage of macromolecules?
12. Suppose there were a hypothetical congenital defect in which the part of the uriniferous tubule that is derived from the ureteric bud failed to unite with the part derived from metanephric tissue. At what point along the tubule system would the discontinuity be?