LABORATORY 17
LIVER, GALL BLADDER, & PANCREAS

OBJECTIVES:

At the end of this lab, you should be able to:

1. identify liver, gall bladder and pancreas by LM and TEM
2. identify the following in the liver:
   - hepatocytes
   - central vein
   - portal triad (portal vein, hepatic artery & bile duct)
   - sinusoids
   - Kupffer cells
   - location of the space of Disse

3. diagram the three different types of liver lobules and describe how each helps to explain some aspect of liver function
4. correlate the ultrastructure of hepatocytes with their functions; identify the space of Disse in electron micrographs and distinguish between the endocrine and the exocrine surfaces of the hepatocyte
5. distinguish the islets of Langerhans from the pancreatic acinar cells and from pancreatic ducts using light microscopy
6. list the cell types found in the islets of Langerhans and the hormone produced by each
7. correlate the ultrastructure with the function of pancreatic acinar cells.
8. explain how the structure of the gall bladder reflects its function, and describe the structural changes that occur when the organ becomes more active

LABORATORY:

Please study the following slides in your set:

Slide 66 (HU Box): Liver, Pig, Mallory Trichrome
Identify a classical liver lobule. It is centered on a central vein and has portal triads at its periphery. The classical lobule is roughly hexagonal in cross section, with a portal triad at each vertex. This section has been stained using the Mallory trichrome to demonstrate the connective tissue, which surrounds each classical lobule in pig liver (see Wheater, Fig. 15.7b, p. 281). In human livers there is normally much less connective tissue, and the boundaries of a classical lobule are much less obvious.

Slide 68C and 68D: Liver, H&E
Identify a classical lobule in these slides of human liver. Locate the central vein in the middle of the lobule and the portal triads around the periphery. Each portal triad contains at least one branch of a bile duct, hepatic artery and portal vein. The bile duct
is lined by a simple cuboidal epithelium and is relatively easy to identify. How can you distinguish the portal vein from the hepatic artery? (Answer: The portal vein has a larger diameter and a relatively thinner wall than the hepatic artery that accompanies it.)

Each portal triad lies in a space called a portal canal, which is bordered by an almost continuous layer of hepatocytes called a limiting plate. Branches of bile duct and vessels in the portal triad must pass through the limiting plate to enter the liver lobules.

Examine the parenchymal cells (hepatocytes) and the sinusoids. What two cell types line the sinusoids? [Answer: Endothelial cells and Kupffer cells (which are macrophages) are intermixed in a single layer that forms the lining of the sinusoids.] Where is the space of Disse located? (Answer: It lies between the sinusoid and the hepatocytes. Therefore it surrounds each sinusoid completely, like a sleeve.) Normally this space is not easily visualized by light microscopy, but in some versions of these slides it is artifactualy enlarged.

In some of these slides the hepatocytes contain a golden brown pigment called lipofuscin. It represents indigestible lipid-derived substances contained within residual bodies. The residual bodies tend to congregate in the area of hepatocyte cytoplasm adjacent to the bile canaliculus. Thus, if the pigment is visible, it serves as a handy marker that tells you a canaliculus is nearby. What makes up the wall of a bile canaliculus? (Answer: Only the plasma membranes of adjacent hepatocytes. The membranes are joined together by tight junctions to form tubular channels between adjacent hepatocytes.) The classical lobule emphasizes the flow of blood, which runs from the portal vein and hepatic artery at the periphery of the lobule toward the central vein. In contrast the portal lobule emphasizes the flow of bile from hepatocytes toward the bile ducts. The portal lobule is roughly triangular in cross section, with a portal triad at its center and a central vein at each of the three vertices.

An acinus of Rappaport (hepatic acinus) has an oval or diamond-shaped cross section with a central vein at both ends of one axis and a portal triad at both ends of the other axis (Ross, Fig. 18.6, p. 635). It can be divided into three concentric zones centered around the axis defined by the portal triads. Hepatocytes within the same zone receive qualitatively similar blood supplies in terms of oxygen, nutrient or toxin content. The acinus helps explain patterns of hepatocyte degeneration seen in some pathological states.

**Slide 68A: Liver Reticulum**

This section was stained with silver salts to demonstrate the reticular fibers that form the hepatic stroma. Most of the reticular fibers are located in the space of Disse, and are produced by Ito cells (stellate cells).

**Slide 67 (HU Box): Liver, Stellate Reticuloendo. Cells (Kupffer Cells), India Ink**

Kupffer cells that have phagocytized particles of India ink prior to fixation look like specks of dirt under low magnification (Wheater, Fig. 15-9, p.282). Kupffer cells and the nonphagocytic endothelial cells both line the lumen of the liver sinusoids. Kupffer cell processes may also extend across the sinusoid so that the cell partially blocks the lumen and is in an excellent position to trap any particulate material passing through the vessel.
Slide 8 (HU Box): Liver, Rabbit, Glycogen, Best’s Carmine

In this section the glycogen in the hepatocyte cytoplasm has been stained red by the Best’s carmine stain. Liver is a major site for storage of excess glucose in the form of glycogen. The glycogen can later be broken down into glucose and released into the blood to maintain blood glucose levels.

Slide 68 (HU Box): Liver and Gall Bladder, or Slide 68E: Liver and Gall Bladder

Note the close relationship between the two organs. Over part of their surface they are in direct contact, with no intervening mesothelium. Note the Kupffer cells of the liver in Slide 68HU. Study the organization of the gall bladder. Identify the simple columnar epithelium that lines the lumen. Gall bladder can be distinguished from other organs that are lined by simple columnar epithelium by examining the epithelial cell type(s) present in each. In gall bladder the epithelium contains one major cell type, the absorptive cell. What epithelial cell type(s) characterize the simple columnar epithelium lining the intestine? (Answer: There is a mixed population of goblet cells and absorptive cells.) What about the stomach? [Answer: There are mainly surface mucous cells, recognizable by the accumulation of mucous granules in their apical cytoplasm (the “apical cup”].

Slide 69: Gall Bladder

The mucosa of the gall bladder may be thrown into deep folds when the gall bladder is empty. It consists of an epithelium and a lamina propria. The striated border of the epithelial cells is usually difficult to see by light microscopy. It may be visible in versions of this slide that are labeled “Gall bladder human cs H5.76” or Gall bladder H&E”. What specialization of the apical membrane makes up this and all other striated borders? (Answer: Microvilli)

Confirm that the gall bladder has no muscularis mucosae and therefore no submucosa. The only muscle layer present is the muscularis externa (often simply called the muscularis). Over most of its outer surface the gall bladder has a serosa, but where the gall bladder adheres directly to the liver, there is an adventitia. (The adventitia/serosa are missing from some slides.)

Slide 70: Bile Duct

Different versions of this slide show the bile duct in cross or longitudinal section. Observe that the bile duct is lined by a simple epithelium composed of tall columnar cells similar to those of the gall bladder. In some versions of Slide 70 the bile duct can be seen passing close to or through pancreatic tissue, near the point where it opens into the duodenum. It is not necessary to identify the layers of the wall of the bile duct.

Slide 65 (HU Box): Pacinian Corpuscle, Pancreas
Slide 71A, 71B or 71C: Pancreas

Observe the pancreatic acinar cells. These are highly polarized cells, with extensive RER at their basal ends, a well-developed supranuclear Golgi that may be visible as a pale area, and numerous secretory vacuoles in the apical cytoplasm.

The acinar cells represent the exocrine portion of the pancreas, and are therefore connected to a duct system. The products of the acinar cells (mostly enzymes) are
carried by these ducts to the duodenum where they aid in digestion. The duct system begins with the intercalated ducts that are located within pancreatic lobules. The first portion of an intercalated duct is made up of centroacinar cells, so called because they bulge into the lumen of the secretory acinus. Centroacinar cells are large and pale compared to acinar cells (see Wheater, Figs. 15.15b, p. 288). They are unique to the pancreas, but are somewhat difficult to locate. Make sure you are not just looking at the pale apical end of a pancreatic acinar cell. Note that none of the intralobular ducts are striated. Pancreas does not contain striated ducts. Next find the large interlobular ducts in the connective tissue septa between lobules.

The endocrine components of the pancreas are the pancreatic islets (islets of Langerhans). At least four cells types are present in the islets. They are difficult if not impossible to distinguish from one another in H&E preparations. Review the major cell types and their functions. One version of Slide 71C (labeled Pancreas Bouin C.H.A.P.) has been specifically stained to demonstrate the islets, which stain royal blue in comparison to the magenta color of the counterstain. Be sure you can distinguish the islets from intralobular ducts.

Some slides contain Pacinian corpuscles. Recall that these receptors mediate deep pressure sensation and vibration.

Acinar cells somewhat resemble the serous cells of the parotid gland, but there are a number of ways to distinguish between pancreas and parotid. The easiest is to look for the islets of Langerhans, which are not found in parotid. Also, pancreas lacks the striated ducts that are characteristic of parotid, and parotid has no centroacinar cells in its intercalated ducts.

**ELECTRON MICROSCOPY (RHODIN)**

A. LIVER

1. Study the diagram (Fig. 30-5) of the blood supply to a classical lobule. Identify branches of hepatic artery, branches of portal vein, sinusoids, central vein and sublobular vein. Trace the direction of blood flow.

2. Study the ultrastructure of a portal canal (Fig. 30-6). This is the space whose outer limit is defined by a layer of hepatocytes called the limiting plate. The portal canal contains loose connective tissue in which are embedded the elements of the portal triad (the portal vein, hepatic artery and bile duct branches). The other hepatocytes of the lobule are arranged in plates that radiate out from the limiting plate and are separated from one another by sinusoids.

3. Compare the components of the portal triad with the central vein (Fig. 30-7). The central vein is very thin-walled and typically has multiple sinusoids emptying into it. The central vein is not accompanied by branches of the hepatic artery or bile duct. It typically has less connective tissue around it than is present in the portal canal.

4. Fig. 30-9 shows a cross section of a sinusoid. It is lined by a cell with very few lysosomes, which is therefore probably an endothelial cell rather than a Kupffer cell. Locate the space of Disse and notice that it completely surrounds the sinusoid and separates it from the hepatocytes. The space of Disse contains many microvilli projecting from the surface of the hepatocytes, and also contains reticular fibers (#6) that make up the stroma of the liver. If these fibers are destroyed by disease processes, hepatocyte regeneration is severely impaired.
5. Study the ultrastructure of hepatocytes (Figs. 30-8 & 30-10). Identify bile canaliculi and distinguish them from sinusoids. Notice that the wall of a bile canaliculus is formed by the plasma membranes of neighboring hepatocytes (Figs. 30-8, 30-10 & 30-11). There are tight junctions on either side of a canaliculus. They run parallel with the canaliculus along its entire length, like the seal on a zip-lock sandwich bag, preventing leakage of bile from the canaliculus into the space of Disse (Fig. 30-10). Lysosomes are common near the canaliculi. Many of these are actually residual bodies (#10 in each figure), containing the pigmented material called lipofuscin. When this is visible by light microscopy, it makes a handy marker for the location of bile canaliculi. Fig. 30-8 is particularly nice because it shows canaliculi cut in longitudinal and cross section. Hepatocytes usually contain a well-developed Golgi complex. One of the functions of the Golgi is to conjugate lipids and proteins to form lipoproteins such as very low density lipoproteins (VLDL, Fig. 30-13). The liver also stores excess glucose in the form of glycogen (Fig. 30-10). Glycogen particles are often found near profiles of smooth endoplasmic reticulum (Fig. 30-12). The SER is also important in the detoxification and metabolism of many toxins and drugs by the liver.

B. GALL BLADDER
Observe the ultrastructure of the surface epithelial cells (Fig. 30-19). These cells are absorptive, but do not have as many microvilli as intestinal absorptive cells. Find the tight junctions between the cells near their apical end, and the elaborate folding (plications) of the plasma membrane in the lateral spaces below the tight junctions (Figs. 30-19 & 30-20). When the gall bladder is actively concentrating bile, ions are pumped into these spaces and water follows, causing the spaces to widen. Thus the width of the lateral intercellular spaces can be used as a rough indication of whether the epithelium was actively concentrating the stored bile at the time of fixation.

C. PANCREAS
1. Identify the pancreatic acini in Fig. 30-23, by locating the zymogen (secretory) granules in the apical ends of the acinar cells. Identify the few places where this section has cut through the lumen of the acinus (#2 in the figure).
2. Study the ultrastructure of the pancreatic acinar cell (Fig. 30-24). This is the classic example of a protein-secreting cell. It was in pancreatic acinar cells that much of the early work defining the organelles involved in protein synthesis and secretion was carried out. Note that an acinar cell has an apical end near the lumen, and a basal end resting on the basal lamina of the acinus. These cells are therefore said to be structurally polarized. Identify the extensive rough endoplasmic reticulum in the basal cytoplasm, the well-developed supranuclear Golgi complex, & the zymogen granules. What mode of secretion do pancreatic acinar cells use? (Answer: Merocrine secretion) What are some of the secretory products within the zymogen granules? (Answer: Enzymes that can aid in the digestive process in the duodenum, including trypsinogen, chymotrypsinogen, elastase, lipases, RNAse, and DNAse)
3. Identify the centroacinar cells in Figs. 30-23 & 30-26, and understand their spatial relationship to the acinar cells.
4. Examine the islet of Langerhans in Fig. 30-28. Although it is possible to identify most types of islet cells based on the morphology of their secretory granules, it is only necessary for you to be able to recognize alpha and beta cells. The islets are characterized by an abundant blood supply. What type of capillary would you expect to find? (Answer: Like most endocrine glands, the islets have fenestrated capillaries. These are more permeable than continuous capillaries, and are therefore able to pick up the pancreatic hormones more easily.) Observe that although the islets cells are not as obviously polarized as the acinar cells, they do have some degree of morphological polarization in that the secretory granules tend to be oriented toward the nearest capillary. A single islet cell often secretes into several surrounding capillaries, i.e., the cells have multiple secretory surfaces.

LABORATORY 17 CHECKLIST
LIVER, GALL BLADDER & PANCREAS

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<th>LIGHT MICROSCOPY</th>
<th>ELECTRON MICROGRAPHS</th>
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<td>reticular fiber stroma</td>
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<td>portal lobule</td>
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<td>gall bladder</td>
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<td>mucosa &amp; muscularis of gall bladder</td>
<td>pancreatic acinar cell</td>
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<td>3 components of a portal triad</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 17: LIVER, GALL BLADDER AND PANCREAS

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 pancreatic acinar cells often exhibit basal basophilia and apical acidophilia?

2. If a patient had no functional pancreatic islet cells of any type, why would you need to provide insulin replacement therapy but not glucagon or somatostatin replacement?

3. Describe the structural changes in gall bladder epithelium that occur when the organ becomes more active.

4. Compare the blood carried by the portal vein and the hepatic artery in terms of oxygen and nutrient content.

5. In addition to carrying nutrient rich blood from the GI tract to the liver, the portal vein also carries blood from the pancreas and spleen. Why is it advantageous to have this blood delivered directly from these organs to the liver?

6. It can be said that in the liver, blood and bile flow in opposite directions. Describe the pathways of blood and bile flow through a classic liver lobule.

7. What is the significance of the space of Disse?