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IFM MICROSCOPIC ANATOMY
COURSE INFORMATION

I. OBJECTIVES:
The IFM Microscopic Anatomy course is taught by the Department of Neurobiology & Anatomy. The objectives of the course are to:

1. Teach the structure of cells, tissues & organs at the microscopic level, with an emphasis on clinical relevance.
2. Demonstrate the principle that structure reflects function.
3. Conceptually connect the molecular events discussed in Biochemistry and Physiology to the macroscopic structures studied in Gross Anatomy.
4. Provide students with the necessary background for Pathology.
5. Prepare students for Board Exams.
6. Foster the development of professionalism among students.

II. FACULTY:
Since almost all the instructors involved in teaching Microscopic Anatomy have offices right here in the Queen Lane building, we do not have official office hours. Feel free to drop by our offices any time you have a question. If we cannot see you immediately we will be happy to make an appointment. E-mails are also always welcome.

<table>
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*Course Director: Questions related to course administration should be directed to Dr. Smith.
#Co-Director: Questions related to laptops/ipads and the course website should be directed to Dr. Goldman.

Departmental Chairman: Dr. Itzhak Fischer
Departmental Office: 272 Queen Lane,
Phone: (215) 991-8401
Departmental Fax: (215) 843-9082
III. REQUIRED BOOKS:

**Text:**  

**Atlas:**  

THE TEXT AND ATLAS SHOULD BE BROUGHT TO EVERY LAB to help you identify and locate the structures you are studying that day.

**NOTE:** If you choose to use books other than these, you do so at your own risk. In the event of any discrepancies between books, the information presented in these two books will be considered correct.


This book is used in all of our lab sessions, but it is now out of print and unavailable from the publisher. Each lab group has one copy of the book in their group locker. We recommend studying and discussing the micrographs together as part of your lab exercise. Additional copies are on reserve in the library. An electronic version of the text is available through the course website.

IV. RECOMMENDED OPTIONAL READING:

During the first two modules of the course there are recommended optional readings from this book, which is on reserve in the library:


V. ADDITIONAL REFERENCE BOOKS:

Consult these in the library if you need additional information on a particular topic.

**Textbooks:**  


**Atlases:**  

VI. COURSE WEBSITE:
You can access the course website by going to:
http://webcampus.drexelmed.edu/IFM
and clicking on “Courses” in the left column and then on “Course Web Site” under Microanatomy in the main page. Parts of this website are password protected.
Alternatively, you can gain access directly to the course website by going to:
http://neurobio.drexelmed.edu/education/ifm/microanatomy

The course website contains items such as the course schedule and all other information found in this syllabus, plus the complete lab manual, fully annotated practice questions for the written exams, a Microscopic Anatomy vocabulary list with definitions, the virtual microscope with digitized glass slides for each lab, a link for electron micrograph images, photos of the faculty, and much more. Photos of the student lab groups will also be posted after the course begins.

VII. OTHER STUDY RESOURCES:
The following resources are available in the lab:

1. **A VIDEOEDISK** (laserdisk) called Histology: A Photographic Atlas, which contains thousands of light microscope images. There are several videodisk players and monitors equipped with this disk in every lab cubicle. At each of these stations there is a 3-ring binder with lists of images that can be called up on the monitor by entering an image number using a remote control device. In addition, each lab group receives one copy of a manual that describes these images in more detail, and includes practice practical questions. **VIEWING AS MANY DIFFERENT EXAMPLES OF A TISSUE OR ORGAN AS POSSIBLE IS AN EXCELLENT WAY TO LEARN ITS STRUCTURE AND TO STUDY FOR PRACTICAL EXAMS. THE VIDEOEDISK PROVIDES YOU WITH THIS OPPORTUNITY.**

2. **A COMPUTER INDEX FOR THE VIDEOEDISK.** Each cubicle has a computer connected to one of the videodisk set-ups. The index program is loaded on this computer. It allows students to view **RANDOMLY MIXED IMAGES** from several study units, which is useful in studying for practicals. Lab instructors will demonstrate the use of this program during the first two or three lab sessions of the course.

The following resource is available in the library:

3. A videodisk set-up. This is especially helpful in studying for exams once the lab has closed for exam set-up.

VIII. LECTURE NOTES:
The lecture notes for each module will be posted online on the IFM website: http://webcampus.drexelmed.edu/ifm/IFM2014/default.aspx. Click on “Modules” in the left column, then click on the name of a module, scroll down to the lecture of interest, and click on the page icon next to the lecture title.

Microscopic Anatomy handouts generally **DO NOT INCLUDE MICROGRAPHS OR DIAGRAMS.** This is quite different from other courses you will be taking that do reproduce many of their figures in their handouts. The reason for this difference is that those courses usually do not use a required textbook and hence they must provide all necessary information in the handout itself. Microscopic Anatomy does have a REQUIRED TEXT AND ATLAS, and you will find many excellent graphics there.
The handouts and the lectures DO NOT contain all that you need to know about the lecture topic. The text, the atlas & the lab manual contain additional information. You are expected to read and learn this material, and IT WILL BE INCLUDED ON EXAMS.

Full handouts ARE NOT PROVIDED for the four Pathology Correlates lectures, which are presented by a guest lecturer, Dr. Cheryl Hanau, chair of the Department of Pathology. Listening to a lecture and learning how to take your own notes is an essential skill that you will need throughout your career as a physician. We offer you the opportunity for some practice during this informal series of lectures.

IX. LAB:

Lab manual: Your lab group will receive one hard copy of the lab manual, which describes in detail the material to be covered in each lab session. It should be kept in your group locker unless all the members of the group agree otherwise. The lab manual is also available online on the course website. Please PREVIEW THIS MATERIAL BEFORE LAB.

Attendance: Attendance at labs is not mandatory. What this means is simply that failure to attend labs will not result in a student receiving a professionalism citation from the course director. HOWEVER, all except the first few labs do include either a one-point lab quiz or a five-point scavenger hunt. Students who do not attend lab lose these points. We include these attendance incentives in the course because we believe that students do benefit from having the expertise of faculty instructors available as they learn. You are strongly urged to attend lab regularly with your lab group.

Peer evaluation: At about the mid-point of the course you will be required to complete an online peer evaluation of your lab partners and yourself. You will be asked to evaluate criteria including “meets group’s expectation of attendance, is consistently well prepared, does his/her fair share (of lab work), respects others, gives and accepts feedback”, and others. You should keep these criteria in mind as you progress through the course.

If you cannot attend a lab session you are expected to notify your lab partners to that effect as soon as possible. Failure to do this often results in poor peer evaluations, and can lead to a professionalism citation if the behavior is repeated in other courses.

Lab access: The laboratory should normally be open 24 hours a day, 7 days a week. If it is not, go to the security desk in the main lobby, show your student ID & ask them to unlock the lab. The only exception to this rule is that the lab will close at noon the day before a practical exam to allow the faculty to set up the exam for the following morning.

Lab equipment: Laboratory equipment (videodisk players and monitors, videodisks, microscopes, computers, etc.) may not be removed from the lab at any time, especially not in the days immediately preceding an exam. Failure to follow this rule will be considered an Honor Court violation.

X. LAB QUIZZES:

The only quizzes in this course are the one-point lab quizzes. They will be given in most labs that do not include a scavenger hunt. They are usually based on one or more light micrographs, electron micrographs, or diagrams that will be projected in lab cubicles. Each lab group also receives a printed copy of the image(s) and the questions (usually 4
or 5). These are CLOSED-BOOK exercises in which group members collaborate, but may not consult any resources other than their lab partners. Answering the majority of questions correctly earns each member of the group one bonus point toward their course grade. Lab quizzes are given at random times during the labs (not always at the beginning or the end). They may cover material that you have just learned during that lab, or material from the previous lab. Students who have not attended the portion of the lab session that preceded the quiz are not eligible to take the quiz. Phoning or texting absent group members to alert them that a quiz is imminent is a violation of the Honor Code.

Lab quizzes may not be made up in cases of absence.

XI. SCAVENGER HUNTS:
There will be 4 scavenger hunts in this course. The date for each is published in the course schedule. A scavenger hunt is a lab exercise in which each lab group is assigned five structures to locate on glass slides from their slide boxes. Students should confer with other members of their lab group. They may use outside resources including textbooks, atlases, lecture notes, lab manual, and videodisk images. The only resource that MAY NOT be used is the virtual microscope images on the course website, since these should be very similar or identical to the glass slides that the group will be using in the scavenger hunt. When your group agrees that the correct structure has been located, you show it to a lab instructor. Each of the students in the group receives one point toward their final grade for each structure correctly identified. Thus each scavenger hunt is worth 5 points and is really the equivalent of a collaborative OPEN-BOOK lab quiz.

A missed scavenger hunt can be made up provided that the course director has been notified prior to the date of the lab, and has excused the absence.

XII. WRITTEN MODULE EXAMS:
There will be 5 written module exams during the course.

All material, whether presented in lecture or in lab, can be tested on a written exam. Material from your required reading, even material that was not specifically discussed in lecture or lab, can be tested on the exams. Questions are in either the MULTIPLE CHOICE OR MATCHING FORMAT. They may include DIAGRAMS.

See the course schedule for the date and time of all written exams and practical exams, and for information about what material is covered in each. Do not email the course director to ask for this information.

See the IFM website http://webcampus.drexelmed.edu/IFM for the official Year 1 exam policies.

Answers to written & practical exams are posted on the same day as the exam. They are posted on the bulletin board in the hall outside the lab (the physical bulletin board, not an online bulletin board). The answers will remain posted until shortly before the next gross Anatomy or Microanatomy exam. Written exams will be scored by the Division of Medical Education, & your grade will be posted on the IFM evaluation website (http://webcampus.drexelmed.edu/evaluations/) in a secure form accessible only by you. Scores are usually posted on the same day as the exam or the following school day.

Challenges to any exam question should be sent by e-mail to the course director (janet.smith@drexelmed.edu) within three school days after the answers have been posted. Any resulting changes in the answer key will be posted on the bulletin board outside the lab.

9
Students may keep the module exam booklets that contain Microscopic Anatomy questions. Since you may find them useful in studying for the cumulative final or for the National Board exam, you should be sure to RECORD THE CORRECT ANSWERS on them and then KEEP THEM IN A SAFE PLACE. Additional copies of the exam will not be available. Answer keys ARE NOT POSTED ONLINE, and the answers will not be made available again once the exam has been removed from the hall bulletin board.

The Microscopic Anatomy faculty also presents lectures and a lab covering the histology of the eye and ear as part of the Neuroscience course. Exam questions related to these topics count toward your Neuroscience grade rather than your Microscopic Anatomy grade.

XIII. PRACTICAL EXAMS:
There will be 5 practical exams during the course. Most practicals include material from two or more modules. Consult the course schedule for the dates and times of the exams and the material covered by each.

Most practicals are walk-around exams that are given in the lab. The course website has a “MAP” OF ALL SIX LAB CUBICLES to illustrate a typical layout for the stations in a practical exam. Click on “Practical Exam Information” in the left column of the course main page.

Test materials can include GLASS MICROSCOPE SLIDES, VIDEODISK IMAGES, DIAGRAMS, & PRINTED COPIES OF LIGHT MICROGRAPHS OR ELECTRON MICROGRAPHS. There will be electron micrographs on all practicals. This is especially true of the first practical, which includes the cell biology portion of the course.

Most practicals consist of 40-45 questions. The questions are in the fill-in-the-blank or multiple-choice format. During lab practicals, students walk from station to station and answer a question related to each image that is presented. Questions often have two parts. The first may be a straightforward identification, whereas the second part may ask something about the function, location, etc. of that structure. You have one minute per station. At the end of each lab practical you are given 10 minutes to revisit any stations you desire.

The course website includes a PRACTICE PRACTICAL FOR THE FIRST EXAM, so that you will have some idea of the types of questions asked on practicals.

The CHEST PAIN PRACTICAL is an exception to this pattern in that we will attempt to deliver this exam (written and practical portions) online. If this test is successful, we may consider giving the two remaining practicals in the course online as well.

Practicals can include a small amount of RELATED MATERIAL FROM A PREVIOUS EXAM. For example when one organ closely resembles another one that was studied earlier in the course, we can use either organ on the practical and expect you to be able to distinguish between them. The images used in practical exams will include some you have seen in lecture or lab, and some you have never seen before. The object of your studies should be to learn how to recognize the various cells, tissues and organs, not to memorize individual slides and micrographs.

The course website contains SLIDE REVIEWS for each practical exam. It is VERY HIGHLY RECOMMENDED that you watch these reviews at least once before taking the exam. Some students also find them useful as previews of what they should be learning during each module.
If you need additional help in preparing for a practical exam, feel free to make an appointment with any faculty member for an EXTRA HELP SESSION IN THE LAB. If you need help with lecture material, please contact the faculty member who gave the relevant lecture(s).

The lab will close shortly after noon on the day before each lab practical to allow the faculty to set up the exam. It will remain closed until several hours after the exam. In addition, PIL or second year classes may be scheduled for the lab on reading days, so all cubicles of the lab may not be available for your use on those days. Please keep this in mind when scheduling your study time.

Answers for the practical will be posted on the same day as the exam on the bulletin board in the hall outside the lab. Practical exams are scored by hand. This process requires considerably more time than scoring the written exams. Therefore practical grades are often not available until several days after the scores for the written exam have been posted. Once the grading is completed, scores for practical exams will also be posted on the IFM evaluations website.

XIV. CUMULATIVE FINAL EXAM

The cumulative final exam is a “MUST PASS” EXAM, meaning that if you do not earn a passing grade on this exam, you will have to take and pass a REMEDIATION EXAM, even if your overall course average is passing. The cumulative final exam is a WRITTEN EXAM ONLY; it has NO LAB PRACTICAL COMPONENT. Questions are in the MULTIPLE CHOICE OR MATCHING FORMAT. NUMEROUS DIAGRAMS are included but there are no light micrographs or electron micrographs. Many of the diagrams come from your required textbook. The exam has 100 questions, each worth ONE HALF POINT toward your course grade. This is the only exam where each individual question is not worth a full point.

XV. MISSING AN EXAM & MAKING UP EXAMS: THE HONOR CODE

Students who cannot take an examination as scheduled must notify the Associate Dean for Student Affairs, Dr. Amy Fuchs, prior to the test. If the Dean is satisfied that the absence is unavoidable, the student will be allowed to take a make-up examination. If the Dean does not excuse the absence, or the student fails to contact the Dean prior to the exam, a grade of zero may be given.

It is our practice in Microscopic Anatomy to use the same exams for make-ups as for the original exam. We expect compliance with the Honor Code. Students who have been excused from an exam and have not yet taken the make-up should not read answers that are posted on the bulletin board, and should not discuss the exam with those who have already taken it.

Students who are excused from a written exam will be informed of the make-up date and time via an email from Cynthia Books, Program Coordinator for Year 1 IFM. Make-ups for excused practical exams must be scheduled with the course director, Dr. Smith janet.smith@drexelmed.edu. It is the student's responsibility to do this as soon as possible.
XVI. GRADING:
Your grade in Microscopic Anatomy is determined by your performance on lab quizzes, scavenger hunts, practical exams, and written exams (including the cumulative final).

The total number of points available in the course will be approximately 450. This includes the scavenger hunts (20 points) and the cumulative final exam (50 points), with the remainder of the points divided between practical exams and the written module exams (probably slightly more written questions than practical). It does not include lab quizzes, because lab quizzes are bonus points (see below).

BONUS POINTS: Bonus points are ones that will be added to your point total if you get them correct, but you will not lose any points if you get them wrong. There are two sources of bonus points in the course:

- The one-point lab quizzes
- Most practical exams will include one or more bonus points

PLEASE remember: Scavenger hunts, written module exams, practical exams, and the cumulative written final exam are REGULAR COURSE POINTS. If you get one of these wrong, you lose a point from the possible course point total.

Grade cut-offs: Decisions about the cut-off points for final grades are made after the course has ended. However, the following guidelines will apply:

- Final averages below 65% are likely to be Unsatisfactory.
- Averages between 65-70% are likely to receive a Marginal Unsatisfactory.
- An average of 70% or above will definitely be a passing grade, providing that the cumulative final is also passed.
- A grade of Highly Satisfactory will only be given to a small number of students who very narrowly miss getting Honors.
- An average of 90% or better will definitely receive Honors, providing that the cumulative final is also passed. This will not change even if the entire class scores above 90%. If you master 90% or more of the material we present, we feel that you deserve Honors, no matter how many other people do the same.

XVII. REMEDIATION:
Students who receive a grade of Unsatisfactory (U) or Marginal Unsatisfactory (MU) in Microscopic Anatomy and those who pass the course but fail the final exam are referred to the Preclinical Promotions Committee for review of their academic record. If the student has few or no deficiencies in other courses, the deficiencies in Microscopic Anatomy can usually be remediated as follows:

- Students who receive a U must take and pass an approved summer course in Microscopic Anatomy or repeat our course the following year. A remedial summer course is offered online by our department, and residential summer courses are usually available at one or more other medical schools.
- Students who receive an MU and those who pass the course but fail the final exam must take and pass a written remediation exam. The exam must be successfully completed no later than two weeks before first year classes begin in August. A student who fails the remediation exam late in the summer, when remediation courses are no longer available, cannot progress into the second year of medical school. Students who need remediation should consult with the course director as soon as possible after the end of the course.
XVIII. EXEMPTION FROM THE COURSE:

Exemption from the course can be granted to students who, within the past 5 years, have earned an average of “B” or better in:

- a Microscopic Anatomy course at an accredited medical school, OR
- a graduate level Microscopic Anatomy course taken while enrolled as a post-baccalaureate student at an accredited college or university

Requests for exemption must be made by e-mailing the course director at janet.smith@drexelmed.edu by the end of the second week of classes (Friday, August 22). Some supporting materials such as a course schedule, syllabus, lab manual, or copies of exams may be required to help evaluate the content of the course you took. You may wish to bring these items with you when you arrive on campus for the start of school. Until a decision is made on the exemption request, students must continue to take all exams. If the request is approved, students will have a grade of "Exempt" recorded on their transcripts.
HOW TO STUDY MICROSCOPIC ANATOMY

I. BEFORE LAB

READ THE TEXTBOOK & ATLAS!
- Unlike several other courses you are taking this year, Microscopic Anatomy has a required text and a required atlas. Read them and look at all the figures. You are responsible on the exams for the material in the text and atlas, even if it has not been specifically covered in lecture or lab.

PREVIEW MATERIAL BEFORE LECTURES AND LABS
- Read or briefly look through the assigned readings in the text before each lecture so that you know what material will be covered and how it is organized. Before each lab, read the lab manual and look at the underlined structures and the checklist. These are the structures you will be finding during that lab session.

BRING YOUR TEXT AND ATLAS TO EVERY LAB
- Use them to help locate the structures you are supposed to be identifying.

II. DURING LAB

During lab your group should work together to study the following:

VIRTUAL MICROSCOPE SLIDES ON THE COURSE WEBSITE:
- For each slide that is to be studied that day, have one member of your group click on each annotation and READ IT ALOUD.
- DO NOT STOP THERE! This is very important! After you have looked at each of the features illustrated in the annotations, try to FIND ANOTHER EXAMPLE OF EACH feature on the same slide. This adds an element of active learning and lets you estimate how well you have understood the annotations that you have read.

GLASS SLIDES FROM YOUR SLIDE BOXES:
- Find the assigned glass slides in your slide boxes, and read the descriptions of each in the lab manual.
- Find examples on the slide of all the underlined structures.
- NOTE: DO NOT NEGLECT THE GLASS SLIDES, They are valuable for demonstrating the normal variability of morphological features. The same structures that you identified on the virtual slide may look significantly different on the glass slide if, for example, the section has been cut from a different tissue block or stained with a different stain. never assume that you have learned all that you need to know from the virtual slides alone.
- If your box doesn’t have a slide that you need, borrow it from another lab group.

VIDEODISK (LASERDISK) IMAGES:
- The videodisk called Histology: A Photographic Atlas contains light microscope images that supplement our glass slide collection. Looking at many different examples of the same tissue (different stains, different magnifications, etc.) is very helpful in learning to recognize structures and distinguish between those that are similar in appearance. IT IS EXCELLENT PREPARATION FOR PRACTICAL EXAMS.
The detailed operation of the videodisk equipment is described in a subsequent section. For now, be aware that you can use the videodisk in one of two ways:

- Use the barcode notebook found at each videodisk station. Using the keypad on the remote control device, type in the barcode number for the image you want to view. There are many images on the videodisk, but little or no explanation accompanies them.


**ELECTRON MICROGRAPHS IN THE RHODIN ATLAS:**

- Near the end of each lab, the manual contains a section titled “Electron Micrographs” that describes selected images from Rhodin’s Atlas of Histology. These micrographs or others similar to them will appear on practical exams. It is to your advantage to study the micrographs with your group during scheduled lab time when instructors are available to answer your questions.

**THE CHECKLIST OF STRUCTURES AT THE END OF EACH LAB:**

- This checklist includes most of the structures that you should be able to identify by light or electron microscopy after you have completed the lab. You can use these lists to assess your mastery of the material and to prepare for exams.

### III. TO STUDY FOR PRACTICAL EXAMS

**For Every Practical:**

- WATCH THE SLIDE REVIEWS for each practical that are posted on the course website.

- MAKE LISTS of structures that could be confused with one another. Know which specific criteria you should use to distinguish one from the other.

**For the First Practical:**

- Use the PRACTICE PRACTICAL posted on the course website to help familiarize yourself with some of the types of questions asked on a practical. This practical covers the material for the first exam (Fundamentals & Abnormal Amniocentesis). There are no practice practicals for subsequent exams because this resource is intended to familiarize you with question formats, not to review factual material.

- Take a look at the PRACTICAL EXAM MAP that is posted online. This shows the typical layout for the stations of a practical exam. Take a few minutes when the lab is empty and walk around the lab, following the path of the exam layout shown on the map. That should make it easier you to orient yourself on the day of the exam.

### IV. TO STUDY FOR WRITTEN EXAMS

- Use the ANNOTATED PRACTICE QUESTIONS on the course website. You can usually expect ~ 3-4 questions/lecture hour on each written exam. The questions are fully annotated with explanations of all correct and incorrect answers.

- Test yourself using the UNANNOTATED WRITTEN EXAM QUESTIONS posted on the course website. These are actual exam questions used in previous years.
V. GET EXTRA HELP IF YOU NEED IT

- Feel free to make an individual or group appointment with any faculty member for extra help with your lab work. We will be glad to meet you in the lab to go over glass slides or the videodisk images. Appointments should be scheduled for no later than one week prior to the next Microanatomy exam.

- For help with lecture material we recommend that you contact the faculty member who gave the lecture. Contact information for each faculty member is listed in the course syllabus and on the course website.

- If you ever have difficulty in arranging for needed help, please contact the course director, Dr. Smith, at: janet.smith@drexelmed.edu
LABORATORY RULES AND REGULATIONS

1. **FEDERAL REGULATIONS PROHIBIT EATING, DRINKING, SMOKING, OR THE APPLICATION OF MAKE-UP IN THE LABS.** When you consider that other courses such as Microbiology, Pathology, & Neuroscience use these labs in studies that involve bacterial cultures, gross pathology specimens, and gross brain specimens, you can see the wisdom of this rule.

2. Laboratory equipment (videodisk players and monitors, videodisks, microscopes, etc.) **may not be removed** from these labs.

3. **The group locker.**
   All the students at one table work as a group. Each group has a locker in the hall outside the lab. Your locker has a ROUND YELLOW sticker with a number that corresponds to your table number in the lab (e.g., locker A3 belongs to Table 3 in cubicle A). Your group should **purchase a combination lock** for your locker and be sure all members of the group know the combination.
   In your group locker you should find:
   a. Two boxes of glass slides (one box of MCP slides and one of Hahnemann slides. The two boxes can be distinguished because the MCP boxes have a number from 1-100 on the side of the box, while the Hahnemann boxes are numbered 200 or higher.)
   b. One copy of the lab manual.
   c. One copy of *An Atlas of Histology* by Johannes Rhodin. This atlas of electron micrographs is used as a part of each lab session. Copies of this book are also on reserve in the library.
   e. One copy of a booklet titled *Hematology*. This will be used during the blood and hematopoiesis lab.
   f. A white iPad to VGA adapter that will allow you to connect your iPad to the VGA cable at your table and thus display an iPad image on your flatscreen monitor for group study.

   **IF ANY OF THESE ITEMS IS MISSING, INFORM THE COURSE DIRECTOR DURING THE FIRST LAB SESSION.**

4. Your group is financially responsible for the items in your locker. At the end of the course, you will be billed for your share of any missing or damaged items. The replacement costs are as follows:
   - Any missing or badly broken slide $5
   - Lab manual $10
   - Review Guide for the videodisk $10
   - Hematology booklet $25
   - VGA Adapter $50
   - Rhodin atlas $175

5. Items from the group lockers should not be removed without first consulting all the other members of the group.

6. There are 3 videodisk players in each cubicle. All the groups in a cubicle must share those three set-ups. **NO INDIVIDUAL LAB GROUP MAY MONOPOLIZE A VIDEODISK THROUGHOUT AN ENTIRE LAB.**
INSTRUCTIONS FOR THE USE OF THE MICROSCOPE

Each lab group has a LIGHT MICROSCOPE equipped with a FLATSCREEN MONITOR. This allows your entire lab group to view the same slide at the same time. We believe you will find the monitor very useful in stimulating group discussions that may answer many of the questions you might be unable to resolve by working alone.

Be sure that everyone learns to use the microscope and monitor, since some of your practical exam questions will be displayed on this equipment. Exam day is not the time to learn how to focus the microscope or adjust the illumination. Detailed instructions are as follows:

Starting-Up:

1. Check that the power strip is plugged into an electrical socket, and that the power strip is turned on (red rocker switch on power strip should be illuminated).
2. Turn on the power switch on the adapter box (the microscope is sitting on top of the square adapter box). One table in cubicle A & one in cubicle B have a different type of set-up and do not have an adapter box. They skip this step.
3. Find the sliding control on the side of the base of the microscope itself. This controls illumination intensity. Set it to “0” before you turn on the illumination source. This prolongs the life of the microscope bulb.
4. Turn on the microscope (by pushing the rocker switch on the base of the microscope next to the illumination intensity control).
5. Increase the illumination with the intensity control to about 2/3 of the maximum. You should see light coming out of the microscope light source. If not, the bulb is probably burned out or a fuse is blown, and you should call an instructor.
6. Turn on the monitor by pushing the “Power” button. The message “Cable not connected” may appear on the screen. Ignore it. Press and hold the “V-Sel” button until the phrase “S-Video” appears on the screen. The screen should be illuminated. If it isn’t, or if the illumination is very dim see “Troubleshooting” below.

Viewing A Slide:

1. Using the coarse focus controls (large knobs on either side of the microscope body), move the stage down (away from the objective lens) & then insert a specimen slide.
2. Find the condenser lens (beneath the stage). Using the knob that controls it (on the right side of the scope under the stage), raise the condenser lens almost as high as it will go toward the stage. It should not be necessary to raise or lower the condenser lens significantly from this position in the normal operation of the microscope.
3. Set the condenser diaphragm wide open (using the flat, black metal lever on the condenser lens beneath the stage).
4. Rotate the objective lens turret until the lowest power objective (4x) comes into position. Always start your examination of any slide at the lowest possible magnification and then gradually increase it.
5. Use the coarse focus controls to raise the stage almost as far as it will go toward the objective lens.
6. Then focus the slide as follows: Look through the microscope eyepieces. Adjust the interocular distance for your eyes by pushing the eyepieces closer together or gently pulling them further away from one another. Still looking through the eyepieces, slowly lower the stage with the coarse focus control and then the fine focus control (small knob at the center of the coarse focus control) until the slide is in focus.

7. If necessary, adjust the illumination until you see an image on the monitor by:
   a. opening or closing the condenser diaphragm with the black lever.
   b. increasing or decreasing the illumination intensity with the slide control on the right side of the base of the microscope.

8. Look at the flatscreen monitor & readjust the fine focus of the microscope if necessary.

9. Look through the eyepieces again, and if necessary focus the eyepieces themselves by turning the movable ring on each eyepiece. The result should be that the image on the monitor and the image that you see through the microscope are both in focus.

10. To change from one magnification to another, rotate the appropriate objective into position while watching to be sure that the objective is not going to hit the slide. Adjust the illumination (see 7a & b above) until you can see the image on the monitor. Then focus using the fine focus knob only. You should not need to make much of an adjustment. Be careful at higher magnifications not to raise the slide up beyond the focus point. If you do that it is possible to crash the slide into the objective lens, destroying both.

   Note #1: The 100X objective is an oil immersion objective. It is labeled "OIL" on the side of the lens barrel or has a black band around the barrel. It should only be necessary to use this objective during the labs on connective tissue and blood. Use of this objective without oil will produce a fuzzy image.

   Note #2: The image seen through the eyepieces of the microscope will always be slightly better than that seen on the monitor. Therefore, if you are having difficulty seeing some fine detail on the monitor, try looking through the eyepieces. You can also see a larger field of view through the eyepieces than is displayed on the monitor.

Shutting-Down:
1. Turn off the power to the monitor.
2. Bring the illumination intensity on the microscope to “0” using the slide control.
3. THEN turn off the microscope.
4. Turn off the rocker switch on the adapter box under the microscope.
5. You may leave the power strip turned on (red rocker switch illuminated).

Troubleshooting:
1. If the monitor screen doesn’t light up when the power strip, adapter box, microscope and monitor are all turned on, check that visible illumination is coming from the microscope light source. If it is not, you may need to increase the illumination intensity using the sliding control, or your instructor may need to replace a burned out bulb or fuse.

2. If the bulb is OK but the monitor screen is still dark or very dim, find the BEAM SPLITTER (the horizontal rod with the black tip, located on the right side of the scope near the eyepieces). It should be pulled all the way out. If it is pushed in, the light is being almost entirely diverted to the eyepieces of the microscope and little or none is reaching the monitor.
INSTRUCTIONS FOR THE USE OF THE VIDEODISK (LASERDISK)

In each cubicle there are three videodisk stations. They are either on the counter around the edge of the room or on mobile stands. LAB GROUPS MUST SHARE THE VIDEODISKS THROUGHOUT THE DURATION OF A LAB PERIOD. Each disk player should contain a copy of the videodisk called Histology: A Photographic Atlas, by Dr. Steven Downing. Detailed instructions are as follows:

Starting-Up:
1. Push the power button on the monitor.
2. Push the power button on the disk player. You should see a blue screen on the monitor.
3. Push the “PLAY” button on the remote control unit or on the videodisk player.
4. Wait while the “PLAY STANDBY” light flashes (or the orange arrow on the videodisk player, depending on what model you’re using).
5. When the flashing stops you should see the title page of the videodisk on the monitor.

Viewing Images:
At every station there should be a notebook listing the images relevant to a particular unit of study and giving the barcode number that corresponds to each image. For example on page 1, the first barcode is for an image of a primary oocyte. To view this image proceed as follows:

1. Enter the barcode number of the image you want to view (37058 for the oocyte), using the number keypad on the black hand-held remote control unit.
2. Press “SEARCH” on the keypad of the remote unit.
3. If you make a mistake entering the number, push “CLEAR” and try again.
4. If you would like to see the barcode number displayed on the monitor along with the image, press “DISPLAY” on the remote unit. This allows you to check that you have entered the correct number and are viewing the intended image. If you want to remove the barcode number and view only the image, press “DISPLAY” again.
5. If you would like to see other related images that may not be listed in the barcode notebook, push “Still/Step Fwd” or “Still/Step Rev” on the remote unit to go through all the images on the disk in forward or reverse order.

Shutting-Down:
1. Push the power button on the monitor to turn off the power.
2. Push the power button on the videodisk player to turn off the power.
HAHNEMANN SLIDE LIST

Guide to stain abbreviations (assume H&E unless otherwise labeled)
   Aldehyde F&M: Aldehyde Fuchsin & Masson Trichrome
   Fe-He: Iron-Hematoxylin
   W.V.G.: Weigert’s van Gieson (a stain for elastic fibers)
   PAS: Periodic Acid-Schiff

<table>
<thead>
<tr>
<th>Slide Number</th>
<th>Slide Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Purkinje Fibers, Human Heart, Mallory Trichrome</td>
</tr>
<tr>
<td>2</td>
<td>Mitosis, Root Tip, I.S.</td>
</tr>
<tr>
<td>3</td>
<td>Auerbach's Plexus, Intestine, Mallory Trichrome</td>
</tr>
<tr>
<td>4</td>
<td>Artery, Vein and Nerve, Elastic Stain, C.S.</td>
</tr>
<tr>
<td>5</td>
<td>Developing Membrane Bone, Human Fetus</td>
</tr>
<tr>
<td>6</td>
<td>Olfactory Epithelium</td>
</tr>
<tr>
<td>7</td>
<td>Bone Marrow, Red, Section</td>
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<td>8</td>
<td>Liver, Rabbit, Glycogen, Best's Carmine</td>
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<tr>
<td>9</td>
<td>Pharyngeal Tonsil, Human</td>
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<td>10</td>
<td>Tonsil, Palatine, Human</td>
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<tr>
<td>11</td>
<td>Tongue, Vallate Papillae, V.S.</td>
</tr>
<tr>
<td>12</td>
<td>Parotid Gland</td>
</tr>
<tr>
<td>13</td>
<td>Salivary Gland, Sublingual, Human</td>
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<tr>
<td>14</td>
<td>Stratified Columnar Epithelium, Human (Salivary Gland)</td>
</tr>
<tr>
<td>15</td>
<td>Esophagus and Stomach, I.S.</td>
</tr>
<tr>
<td>16</td>
<td>Stomach and Duodenum, I.S.</td>
</tr>
<tr>
<td>17</td>
<td>Recto-Anal Junction, I.S.</td>
</tr>
<tr>
<td>18</td>
<td>Epiglottis, I.S.</td>
</tr>
<tr>
<td>19</td>
<td>Lung, Fetal</td>
</tr>
<tr>
<td>20</td>
<td>Mammary Gland, Inactive, Human</td>
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<td>21</td>
<td>Mammary Gland, Active</td>
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<tr>
<td>22</td>
<td>Penis, Human, C.S.</td>
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<tr>
<td>23</td>
<td>Fallopian (Uterine) Tube, Fimbriated End, Human, C.S.</td>
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<tr>
<td>24</td>
<td>Epididymis, Human</td>
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<td>25</td>
<td>Vas Deferens, Human, C.S.</td>
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<tr>
<td>26</td>
<td>Seminal Vesicle, Human, C.S.</td>
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<td>27</td>
<td>Ovary, Corpus Luteum of Ovulation, Human</td>
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<tr>
<td>28</td>
<td>Uterus, Follicular Phase, (Proliferative), Human</td>
</tr>
<tr>
<td>29</td>
<td>Uterus, Progravid Phase, (Secretory), Human</td>
</tr>
<tr>
<td>30</td>
<td>Chondroid Tissue, Human, I.S.</td>
</tr>
<tr>
<td></td>
<td>Description</td>
</tr>
<tr>
<td>---</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>31</td>
<td>Cervix Uteri, Human, l.s.</td>
</tr>
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<td>32</td>
<td>Vagina, Human, l.s.</td>
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<tr>
<td>33</td>
<td>Blood Smear, Wright-Giemsa Stain</td>
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<tr>
<td>34</td>
<td>Umbilical Cord, Human, c.s.</td>
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<tr>
<td>35</td>
<td>Hyaline Cartilage, Human (Trachea)</td>
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<td>36</td>
<td>Fibrocartilage</td>
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<tr>
<td>37</td>
<td>Elastic Cartilage, Elastic Stain</td>
</tr>
<tr>
<td>38</td>
<td>Bone, Ground, Unstained</td>
</tr>
<tr>
<td>39</td>
<td>Membranous Bone, Fetal Skull, Coronal Section, Mallory Trichrome</td>
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<tr>
<td>40</td>
<td>Developing Long Bone, l.s.</td>
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<td>41</td>
<td>Fetus, Rat, Sagittal Section</td>
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<tr>
<td>42</td>
<td>Smooth Muscle, Teased</td>
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<tr>
<td>43</td>
<td>Skeletal Muscle, Entire, c.s.</td>
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<tr>
<td>44</td>
<td>Cardiac Muscle, Mallory Trichrome or H&amp;E</td>
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<tr>
<td>45</td>
<td>Aorta, c.s.</td>
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<td>46</td>
<td>Aorta, Human, Elastic Stain, c.s.</td>
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<td>47</td>
<td>Artery and Vein, c.s.</td>
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<tr>
<td>48</td>
<td>Entire Heart, l.s.</td>
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<td>49</td>
<td>Lymph Node, Human</td>
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<tr>
<td>50</td>
<td>Spleen</td>
</tr>
<tr>
<td>51</td>
<td>Reticular Fibers, Silver Stain</td>
</tr>
<tr>
<td>52</td>
<td>Thymus, c.s.</td>
</tr>
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<td>53</td>
<td>Lip, Skin (Thin)</td>
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<td>54</td>
<td>Skin, Thick</td>
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<tr>
<td>55</td>
<td>Tongue</td>
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<tr>
<td>56</td>
<td>Esophagus, c.s.</td>
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<tr>
<td>57</td>
<td>Stomach, Cardiac Region</td>
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<td>58</td>
<td>Stomach, Fundic Region, Human</td>
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<td>59</td>
<td>Stomach, Pyloric Region, Human</td>
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<tr>
<td>60</td>
<td>Duodenum, c.s.</td>
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<tr>
<td>61</td>
<td>Jejunum</td>
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<td>62</td>
<td>Ileum, c.s.</td>
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<td>63</td>
<td>Colon, Human</td>
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<td>64</td>
<td>Appendix, c.s.</td>
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<td>65</td>
<td>Pacinian Corpuscle, Pancreas</td>
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<td>66</td>
<td>Liver, Pig, Mallory Trichrome</td>
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<tr>
<td>67</td>
<td>Liver, Stellate Reticuloendothelial Cells (Kupffer Cells)</td>
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<td>68</td>
<td>Liver and Gall Bladder, Monkey</td>
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<td></td>
<td>Description</td>
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<tr>
<td>69</td>
<td>Liver, Human</td>
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<tr>
<td>70</td>
<td>Trachea and Esophagus, c.s.</td>
</tr>
<tr>
<td>71</td>
<td>Lung</td>
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<td>72</td>
<td>Lung, Elastic Stain</td>
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<td>73</td>
<td>Kidney, Whole, Coronal Section</td>
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<td>74</td>
<td>Kidney, Human</td>
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<td>75</td>
<td>Urinary Bladder, Distended</td>
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<td>76</td>
<td>Ureter, Human, c.s.</td>
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<td>77</td>
<td>Pituitary Body</td>
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<td>78</td>
<td>Parathyroid, Primate (Thyroid, Parathyroid)</td>
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<td>79</td>
<td>Suprarenal Gland, Human</td>
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<td>80</td>
<td>Testis</td>
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<td>Spermatogenesis, Rat Testis, Iron Hematoxylin</td>
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<td>82</td>
<td>Transitional Epithelium, Human (Urinary Bladder Contracted)</td>
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<td>83</td>
<td>Prostate, Older Human</td>
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<td>84</td>
<td>Penis, Fetal, Masson Trichrome</td>
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<td>85</td>
<td>Ovary</td>
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<td>86</td>
<td>Ovary, Corpus Luteum of Ovulation, C.T. Stain</td>
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<tr>
<td>87</td>
<td>Knee, Rat, Methylene Blue</td>
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<tr>
<td>88</td>
<td>Fallopian (Uterine) Tube, Ampulla, Human, c.s.</td>
</tr>
<tr>
<td>89</td>
<td>Fallopian (Uterine) Tube, Isthmus, Human, c.s.</td>
</tr>
<tr>
<td>90</td>
<td>Vagina, Human</td>
</tr>
<tr>
<td>91</td>
<td>Urethra, Female, c.s.</td>
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<td>92</td>
<td>Pineal Body, Human</td>
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<td>93</td>
<td>Nissl Bodies, Neurocytes, Nissl Method</td>
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<tr>
<td>94</td>
<td>Motor End Organs, w.m., Gold Chloride/Formic Acid Technique</td>
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<tr>
<td>95</td>
<td>Medullated Nerve, c.s. &amp; l.s. PAS-Orange G</td>
</tr>
<tr>
<td>96</td>
<td>Salivary Gland Complex, Rat</td>
</tr>
<tr>
<td>97</td>
<td>Tendon, Human, c.s. &amp; l.s.</td>
</tr>
<tr>
<td>98</td>
<td>Bone, Decalcified, c.s. &amp; l.s.</td>
</tr>
<tr>
<td>99</td>
<td>Skin, Human, Pigmented &amp; Nonpigmented, Elastic Stain</td>
</tr>
<tr>
<td>100</td>
<td>Placenta, Human</td>
</tr>
</tbody>
</table>
# MCP SLIDE LIST

Guide to stain abbreviations (assume H&E unless otherwise labeled)
- Aldehyde F&M: Aldehyde Fuchsin & Masson Trichrome
- Fe-He: Iron-Hematoxylin
- W.V.G.: Weigert’s van Gieson (a stain for elastic fibers)
- PAS: Periodic Acid-Schiff

<table>
<thead>
<tr>
<th>Slide Number</th>
<th>Slide Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mast Cells, Tongue, Toluidine Blue</td>
</tr>
<tr>
<td>1A</td>
<td>Liver, Trypan Blue Safranin</td>
</tr>
<tr>
<td>2</td>
<td>Tendon, Longitudinal Section (LS)</td>
</tr>
<tr>
<td>3</td>
<td>Tendon, Cross Section (CS)</td>
</tr>
<tr>
<td>4A</td>
<td>External Ear</td>
</tr>
<tr>
<td>4B</td>
<td>External Ear, Aldehyde-F&amp;M</td>
</tr>
<tr>
<td>6</td>
<td>Ground Bone</td>
</tr>
<tr>
<td>7</td>
<td>Rib</td>
</tr>
<tr>
<td>7A</td>
<td>Rib</td>
</tr>
<tr>
<td>8A</td>
<td>Bone</td>
</tr>
<tr>
<td>8B</td>
<td>Cancellous Bone</td>
</tr>
<tr>
<td>10</td>
<td>Finger</td>
</tr>
<tr>
<td>11</td>
<td>Bone, Developing</td>
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<tr>
<td>12</td>
<td>Blood Smear, Wright’s Stain</td>
</tr>
<tr>
<td>13</td>
<td>Red Bone Marrow Smear</td>
</tr>
<tr>
<td>14A</td>
<td>Skeletal Muscle</td>
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<tr>
<td>14B</td>
<td>Skeletal Muscle, Fe-He</td>
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<td>17</td>
<td>Spinal Ganglion, Homo</td>
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<td>18A</td>
<td>Osmic Nerve, CS</td>
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<tr>
<td>18B</td>
<td>Osmic Nerve, LS</td>
</tr>
<tr>
<td>22</td>
<td>Tonsil, (Faucial) Pharyngeal</td>
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<tr>
<td>23</td>
<td>Immature Thymus, Newborn</td>
</tr>
<tr>
<td>24</td>
<td>Thymus, Homo 22 yrs</td>
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<td>25</td>
<td>Lymph Gland (Node), Human</td>
</tr>
<tr>
<td>26A</td>
<td>Lymph Node, Homo 13 Years or Homo</td>
</tr>
<tr>
<td>26B</td>
<td>Lymph Node, Reticular Fiber Stain</td>
</tr>
<tr>
<td>27</td>
<td>Eye, Monkey</td>
</tr>
<tr>
<td>28A</td>
<td>Spleen, Reticular Fiber Stain</td>
</tr>
<tr>
<td>28B</td>
<td>Spleen</td>
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<td>28C</td>
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<td>Iliac Artery, Ald. F&amp;M</td>
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<td>Renal Artery, Elastin Stain</td>
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<tr>
<td>34A</td>
<td>Mesentery Spread (Actually a section of mesentery, not a spread)</td>
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<td>Mesentery Spread (Actually a section of mesentery, not a spread)</td>
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<td>Inferior Vena Cava</td>
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<td>Mammary Gland, Homo 28 Years</td>
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LABORATORY 1
MICROSCOPY, STAINS & ORGANELLES

OBJECTIVES:

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

1. identify the parts of a compound light microscope and describe the function of each

2. be able to focus a light microscope and obtain a well-illuminated image at any magnification

3. distinguish by light microscopy between cells, nuclei & nucleoli

4. distinguish between euchromatin and heterochromatin

5. use landmarks and cues within the tissue (such as the size of a red blood cell) to estimate size and distance

6. recognize basophilic and acidophilic structures after staining with hematoxylin and eosin (H & E)

7. recognize smooth muscle vs. connective tissue in a trichrome-stained specimen

8. describe the uses of each of the stains illustrated or discussed in this lab, i.e., know what tissue components are stained by each

9. identify common types of artifacts in tissue sections (folds, tissue shrinkage, dried mounting medium, knife marks, chatter)

10. recognize the major organelles in electron micrographs and understand their structure and function

LABORATORY:

I. THE LIGHT MICROSCOPE

Your primary tool for examining tissues in histology and in pathology will be the compound light microscope. Learn to use it optimally. The basic parts of a light microscope (Fig. 1) are the light source (a bulb in our case), the condenser lens (beneath the stage), the condenser diaphragm, the stage (which holds the slide), the objective lenses (on the revolving turret), and the ocular lens or eyepiece. Adjusting the illumination is normally done by moving the sliding control on the side of the base of the microscope. If necessary the condenser diaphragm can be opened or closed to improve the image.
II. FUNDAMENTAL FACTS

A. Units of Measure in Microscopy
   1. The unit of measurement most often used in light microscopy is the micrometer (µm) also called the micron (µ).
      
      \[
      \text{1 micrometer} = 1 \text{ micron} = 10^3 \text{ nanometers (nm)} = 10^{-3} \text{ millimeter (mm)} = 10^{-6} \text{ meter (m)}
      \]

      Most cells are 10-30 µm in diameter. Sections for routine light microscopy average 5-15 µm in thickness. Thus a single cell is usually visible in several adjacent sections (called serial sections). Sections for electron microscopy average 50-150 nm (0.05-0.15 µm). Thus it can require several hundred electron microscope sections to pass through the thickness of a single average cell.

   2. Red blood cells (erythrocytes) are approximately 7.0-7.5 µm in diameter. They are present in most tissue sections and make very useful built-in rulers for estimating the size of other structures in the section.

B. Visualization Of 3-Dimensional Structures From Slices:

   In determining the three-dimensional image of an object from its appearance in a section 3 things are useful: 1) practice, 2) some prior knowledge of the shape of the structure, and 3) the ability to understand that some structures look very different when cut in different planes. For example, the outline of a cylindrical object would appear to be oval rather than circular if the object were cut obliquely rather than in true cross section (Fig. 2).

![Fig. 2: Effect of plane of sectioning on the appearance of an object. Transverse (Cross) Section (A) vs. Oblique Section (B).]
Another complication occurs when a tangential section passes through the wall of a hollow organ but misses the lumen. The result is that the organ may appear to be solid rather than hollow (Fig. 3).

Fig. 3: Effect of plane of sectioning on the appearance of an object. Tangential Sections.

The effect of the plane of section also depends on the shape of the structure. For example (Fig. 4) a spherical cell will always look round no matter how it is sectioned (Plane A vs. Plane B), but a columnar cell will look very different if it is cross-sectioned (Plane C) vs. longitudinally sectioned (Plane D).

Fig. 4: The effect of sectioning in different planes depends on the 3-dimensional shape of the object.

It is also important to remember that a single section may not include all the parts of a structure. For example (Fig. 5) one section may pass through the nucleus of a cell while another section through the same cell misses the nucleus completely and gives the appearance of an anucleate cell.
III. GLASS SLIDES

It is almost time for you to look at some glass slides. Before you do you should understand the following:

In this manual slides from the Hahnemann collection are indicated by the notation “HU Box” following the slide number, while slides from the MCP collection are indicated by the slide number alone. Thus Slide 20 would be from the MCP box, while Slide 20 (HU Box) would be from the Hahnemann collection.

You do not have to study every slide listed in a lab exercise. If you are given a choice of slides (e.g., “Slide 85 HU or Slide 17”) it is sufficient to study one or the other. However, if you are told to look at “Slide 85 HU and Slide 17”, then you should look at both, because each one shows some feature that the other lacks.

If you don’t have any of the slides you need, try to borrow one from another group. If that fails, you can consult the videodisk, or use your text or atlas to find an example of the structure you are supposed to be studying.

Assume a slide is stained with hematoxylin & eosin (H&E) unless otherwise specified.

The notation “c.s.” on a slide label stands for cross section, while “l.s.” stands for longitudinal section.

Sometimes the slide label also indicates the fixative that was used in tissue preparation. Terms such as Zenker, Bouin, or Helly are names of particular fixatives.

YOU WILL FIND A SLIDE LIST FOR THE HU BOXES AND MCP BOXES ON PAGES 21-27 OF THIS MANUAL. Use it if you are told to study “any slide showing” a particular tissue or organ. Also, if the slide has been stained with something other than H&E, the slide list will usually identify what stain was used.

Remember that you are responsible for identifying all STRUCTURES THAT ARE UNDERLINED IN THE WRITTEN DESCRIPTION OF ANY GLASS SLIDE OR ELECTRON MICROGRAPH. THERE IS ALSO A CHECKLIST OF THESE STRUCTURES AT THE END OF EACH LAB.
Now study the slides listed below.

Always start by examining any slide with the naked eye before placing it on the microscope. Hold it up to the light or against a white piece of paper. Try to determine the general geometry of the organ. Does it look like a hollow organ cut in cross section (e.g., large airway, blood vessel, intestine)? Is it a solid piece of tissue that may have come from an organ that lacks a lumen (e.g., ovary)? Does the tissue look homogeneous throughout the section (e.g., liver), or are there regional differences (e.g., evidence of layering in the wall of the GI tract)?

Then place the slide on the microscope stage, always beginning at the lowest magnification (4x). Get an overview of the entire slide and identify the region(s) you want to examine at higher magnifications.

In this first group of slides we are concerned with your ability to tell the difference between an entire cell, the nucleus, and the nucleolus. In looking at any cell, you should train yourself to evaluate additional characteristics such as: cell size, cell shape, nuclear shape, location of the nucleus within the cell, whether the nucleus is euchromatic or heterochromatic, the number of nucleoli present (if any), the staining affinity of the cytoplasm (acidophilic or basophilic), and whether there are visible cytoplasmic vacuoles.

A. Cell size:
   Slide 85 (HU Box): Ovary, or
   Slide 17: Spinal Ganglion

   Although the average cell is 10-30 µm, other cell types are much larger. Megakaryocytes in bone marrow, osteoclasts in bone, oocytes in the ovary and some neurons are examples of cells that can be identified in part on the basis of their very large size. In Slide 85HU look for the large oocytes in the secondary follicles and the Graffian follicles of the ovary. Consult your atlas to find examples of what you should be looking for. Alternatively, in Slide 17 find the cell bodies of the large sensory neurons in the spinal ganglion (dorsal root ganglion). Notice that some of these cells appear to lack a nucleus. In reality they all have a nucleus, but the cells are so large that some sections pass only through cytoplasm, missing the nucleus entirely (as illustrated in Fig. 4 above).

   Another important cell as far as size is concerned is the red blood cell (RBC or erythrocyte). Erythrocytes are not particularly large, having an average diameter of about 7 microns, but they are very consistent in size and they are present within blood vessels on almost every slide. Thus they serve as handy internal rulers that can help you estimate the size of other structures on a slide. Find an RBC on one of these slides and use it to estimate the diameter of the oocyte or neuron.

B. Cell Shape
   Slide 42 (HU Box): Smooth Muscle, Teased, or
   Slide 66: Appendix, or any slide of large or small intestine

   Cell shape can also provide clues as to a cell's identity. For example smooth muscle cells and fibroblasts tend to have an elongated shape that is often described as cigar-shaped, spindle-shaped or fusiform. In contrast, more mobile cells such as white blood cells are usually roughly spherical. Different types of neurons often have highly distinctive shapes (bipolar neurons, multipolar neurons, pseudounipolar neurons) that help to establish their identity.
In Slide 42HU smooth muscle cells have been teased apart until the individual cells are separated from one another, making it easier to appreciate their elongated shape. Throughout the course you will hear cells such as these described as “fusiform” or “spindle-shaped. (The cells have been stained with a very nonspecific red dye that stains nucleus and cytoplasm the same color. Therefore you will not usually be able to distinguish nucleus from cytoplasm in this slide.)

Slide 66 shows the circular and longitudinal layers of smooth muscle in the wall of a cross-sectioned appendix. The individual smooth muscle cells have the same elongated shape in both layers, yet they look quite different on the slide because the cells in the two different layers have been sectioned in different planes. Find the outer muscle layer. Here the cells are longitudinally arranged with their long axis parallel to the long axis of the appendix. In a cross section of the organ these cells will also be cut in cross section and will appear round. Next find the inner muscle layer. Here the cells are circularly arranged, encircling the lumen of the appendix. In a cross section of the organ, these cells are cut longitudinally so that their elongated fusiform shape will be apparent. These same relationships can be observed in Wheater, Fig. 6.18, p. 112.

C. Nucleus and Nucleolus

Slide 93 (HU Box): Nissl Bodies, Neurons, Nissl Method

The nucleus provides clues that can be used in identifying cells. For example:

1. Is the cell uninucleate or multinucleate? A few cell types such as osteoclasts are characteristically multinucleate, whereas most are uninucleate.

2. Note the shape of the nucleus (round, lobed, etc.). Some cells, such as mature neutrophil or eosinophils have a distinctive nuclear shape (3-5 lobes in neutrophils; usually 2 lobes in eosinophils). In other cells the shape of the nucleus is roughly the same as that of the cell itself (e.g., the elongated shape of the nucleus in a smooth muscle cell).

3. Note the relative amounts of heterochromatin vs. euchromatin. Some cell types such as small lymphocytes are extremely heterochromatic, whereas others such as hepatocytes and neurons are typically very euchromatic. A cell with a heterochromatic nucleus is using only a small percentage of its genome; one with a euchromatic nucleus is using a large percentage.

4. Note the presence, size and number of nucleoli. Nucleoli are the sites of ribosomal RNA transcription. Cells with a high rate of protein synthesis need many ribosomes and therefore tend to have multiple nucleoli or one very large nucleolus. Cells with a very low rate of protein synthesis may have no visible nucleolus at all. Immature white blood cells, for example, start off with multiple nucleoli that disappear as the cells mature and their rate of protein synthesis falls.

Slide 93HU is a section of spinal cord. Find the somatic motor neurons. They are located in what is technically called the ventral horn of the spinal cord (you will learn about that later). For now look at Wheater, Fig. 20.10, p. 392 to see the location of the ventral horn. Look within the ventral horn on your slide, and find the largest cells in that region. These are the somatic motor neurons that innervate skeletal muscle. Be sure you can distinguish between the cytoplasm, nucleus and nucleolus of these neurons. The nuclei are highly euchromatic, while the nucleoli are very dark-staining basophilic structures. Notice also that the cytoplasm of these cells is filled with deeply basophilic clumps called "Nissl bodies." Each Nissl body contains rough endoplasmic reticulum plus free ribosomes. Their abundance suggests a high rate of protein synthesis in these cells.
Next study the following slides to become familiar with the appearance of cells and tissues using different stains:

D. Hematoxylin & Eosin
   Slide 12, 13 or 14 (HU Box): Various salivary glands, or
   Slide 57A, 57B or 57C: Various salivary glands

   Hematoxylin and eosin (referred to as H&E) is the most widely used stain in histology. It is considered to be a “nonspecific” stain that relies on charge interactions with the tissue rather than specific binding to a particular chemical group. Unless otherwise specified, you should assume that the slides in your boxes have been stained with H&E. For routine microscopy, sections are stained first with hematoxylin and then with eosin. Hematoxylin is known as a basic dye while eosin is an acidic dye. Please note that histologists use the terms “basic” and “acidic” in a different way than what you learned in chemistry. Through long-standing convention, histologists call dyes that bind to positively charged tissue components “acidic”, while dyes that bind to negatively charged tissue components are "basic" stains.

   Tissue components that bind basic dyes are said to be basophilic (literally “base-loving”). They stain blue to purple with most basic stains. The most common basophilic components are nuclei (because of the phosphate groups in their DNA), nucleoli and ribosomes (because of the phosphate groups in their RNA), and proteoglycans and glycosaminoglycans (GAGs) (because of their sulfate & carboxyl groups). Proteoglycans & GAGs are abundant in the matrix of certain kinds of cartilage, accounting for its bluish staining with H&E.

   Tissue components that bind acidic dyes are said to be acidophilic (“acid-loving”) or eosinophilic (“loving eosin”). They usually stain pink or red with the acidic dyes. In fixed tissues, most proteins have a net positive charge and will therefore be acidophilic. Thus, organelles with a high protein content such as lysosomes, mitochondria or secretory granules will make the cytoplasm of a cell acidophilic if they are present in abundance. Similarly, the extracellular matrix is often acidophilic since it is rich in proteins such as collagen.

   It is important to note that the basophilia or acidophilia of tissue components depends on the pH at which the staining is carried out since the pH affects the charge of the macromolecules. Most staining is done at a pH where nucleic acids, proteoglycans and GAGs have a net negative charge, while most proteins have a net positive charge.

   Some structures such as Golgi cisternae are neither strongly basophilic nor strongly acidophilic. Therefore the area of the cytoplasm where the Golgi cisternae congregate (called the cytocentrum or centrosome) often appears paler than the rest of the cytoplasm. It also contains the centrioles as well as the Golgi. It is only apparent when the remainder of the cytoplasm is strongly basophilic (due to an abundance of ribosomes) or strongly eosinophilic (due to many mitochondria, lysosomes, etc.)

   Examine one or two of the slides listed above. Identify nuclei. Evaluate the cytoplasm of the secretory cells in the glands. Their cytoplasm is usually basophilic because it contains many ribosomes on the rough endoplasmic reticulum. These ribosomes are producing the secretory proteins released by these glands.

E. Trichrome Stains
   Masson: Slide 34B: Mesentery Spread, or
   Slide 56B: Taste Buds
   Mallory: Slide 66 (HU Box): Liver, Pig, or
   Slide 39 (HU Box): Fetal Skull, Intramemb. Bone Formation
   (CAREFUL!: Some versions of these slides are stained with H&E rather than a trichrome)
Another useful staining method is a trichrome stain. There are many different trichromes including Masson trichrome and Mallory trichrome. A trichrome includes three or more different stains used as a mixture or sequentially. Trichromes were developed to help identify connective tissues by staining collagen a distinctive color (collagen is a major component of most connective tissues). They are especially useful in distinguishing connective tissue from smooth muscle cytoplasm, both of which are acidophilic after H&E staining. Different trichromes stain collagen slightly different colors. For example Masson trichrome stains collagen aqua, while Mallory trichrome stains it a truer blue (sky blue or darker). With both these trichromes most other tissue components (muscle, erythrocytes and most cell nuclei) stain varying shades of orange, red or reddish purple, making it easy to distinguish them from collagen.

F. Silver Stains for Reticular Fibers
   Slide 26B: Lymph Node, Reticular Fiber Stain, or
   Slide 28A: Spleen, Reticular Fiber Stain

   A variety of different silver stains exist. Some are specific for reticular fibers, causing them to stain black. Go to 40x on these slides and find the network of fine black reticular fibers. The cells on this slide are visible only because they have been counterstained with a nonspecific red dye. If a counterstain had not been used, you would not see much more than a network of black reticular fibers.

G. Elastic Stains
   Slide 4B: External Ear, Aldehyde Fuchsin-Masson
   Slide 41: Plantar Skin
   Slide 31B: Iliac Artery, Ald. F & M, or
   Slide 37 (HU Box): Elastic Cartilage

   Elastic fibers stain very poorly with most stains, but there are several specialized elastic stains that make them immediately obvious. One example is aldehyde fuchsin, which stains elastic fibers purple. It is often used with a trichrome, especially the Masson trichrome in a combination called aldehyde fuchsin-Masson (or “aldehyde F&M”). It is the trichrome that has stained the collagen green in the first three slides listed here and the aldehyde-fuchsin that has stained the elastic fibers purple. Note that elastic fibers are not the only things which have stained purple: epithelial cells, smooth muscle and red blood cells have been stained a red to purple color by the trichrome component of this combined stain. In Slide 37HU, the elastic stain has been used in combination with H&E rather than a trichrome.

   Other elastic stains also exist, such as orcein, which stains elastic a dark brown.

H. Carbohydrate Stains
   Slide 8 (HU Box): Liver, Glycogen, Rabbit, Best’s Carmine

   Best’s carmine is a highly specific stain that stains glycogen red. Observe the glycogen deposits in the cytoplasm of the hepatocytes (liver cells). One function of the liver is to take up excess glucose from the blood and store it in the form of glycogen. In well-fed individuals there will be extensive glycogen deposits. After fasting, the amount will be greatly reduced. The nuclei and the rest of the cytoplasm are visible because they have been counterstained with another stain.
Slide 51B: Kidney PAS (This is a particularly rare slide. If you cannot find one, compare Figs. 16.16b-d, p. 305 in Wheater.)

The periodic acid-Schiff procedure (PAS) stains most carbohydrate-rich structures a distinctive shade of magenta. At high magnification (40X) the basement membrane of the kidney tubules and of the glomerular capillaries is visible because of their high carbohydrate concentration. Other PAS positive structures in the body include reticular fibers in connective tissue, the mucous granules of goblet cells, and the microvilli of intestinal epithelial cells and of certain kidney tubules.

I. Lipid Stains
Slide 18A: Osmic Nerve, Cross-Section, and Slide 18B: Osmic Nerve, Longitudinal Section

Find the myelin sheaths of myelinated nerves. They are composed largely of lipid that stains black with osmium stains. Other lipid stains include Oil Red O, which stains lipid red and Sudan Black B, which stains lipid gray to black. Lipid presents a particular challenge since it is easily extracted during tissue preparation unless specialized fixatives are used. If the lipid has been extracted, you will see only empty unstained white spaces where the lipid used to be. Look at Wheater, Fig. 4.15, p. 75, which shows adipocytes in white fat. They contain a single large lipid droplet that fills nearly their entire cytoplasm, but here the lipid has been extracted, and you see the empty white space within each cell where it used to be.

The table below summarizes the stains we have looked at plus a few other that may be referred to from time to time during this course.

<table>
<thead>
<tr>
<th>Stain</th>
<th>Tissue Component(s) Detected</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematoxylin</td>
<td>Basophilic (negatively charged) structures</td>
<td>Bluish purple</td>
</tr>
<tr>
<td>Eosin</td>
<td>Acidophilic (positively charged) structures</td>
<td>Pink/red</td>
</tr>
<tr>
<td>Masson trichrome</td>
<td>Collagen</td>
<td>Aqua</td>
</tr>
<tr>
<td>Mallory trichrome</td>
<td>Collagen</td>
<td>Blue</td>
</tr>
<tr>
<td>Certain silver stains</td>
<td>Reticular fibers</td>
<td>Black/brown</td>
</tr>
<tr>
<td>Aldehyde fuchsin</td>
<td>Elastic fibers, mast cell granules, cartilage matrix</td>
<td>Purple</td>
</tr>
<tr>
<td>Orcein</td>
<td>Elastic fibers</td>
<td>Brown</td>
</tr>
<tr>
<td>PAS (Periodic Acid Schiff)</td>
<td>Most carbohydrates (good for demonstrating mucins, thick basement membranes, thick glycosalyces, reticular fibers)</td>
<td>Magenta</td>
</tr>
<tr>
<td>Best's carmine</td>
<td>Glycogen</td>
<td>Crimson</td>
</tr>
<tr>
<td>Osmium tetroxide</td>
<td>Unsaturated lipids</td>
<td>Black</td>
</tr>
<tr>
<td>Sudan black B</td>
<td>Lipid</td>
<td>Gray to Black</td>
</tr>
<tr>
<td>Oil red O</td>
<td>Lipid</td>
<td>Red</td>
</tr>
<tr>
<td>Methyl green-pyronine</td>
<td>DNA (due to methyl green)</td>
<td>Green</td>
</tr>
<tr>
<td></td>
<td>RNA (due to pyronine)</td>
<td>Pink to Red</td>
</tr>
</tbody>
</table>

37
IV. ARTIFACTS

Frequently, during the preparation of a slide, problems may occur that result in minor artifacts in the tissue section. These must be recognized as artifacts rather than normal structures or evidence of pathology. Examples include folds or tears in the tissue, knife marks (scratches across the section due to nicks in the edge of the knife), chatter (parallel bands of thick and thin regions in the section due to the bouncing of the knife along the surface of the tissue as the section was cut), over-staining or understaining, tissue shrinkage that leaves spaces between structures where none exist in life, precipitates from the fixative or stain, etc. When you encounter something unusual in the course of your studies, remember the possibility that it may simply be an artifact. It is not essential that you study examples of all these artifacts at this point, but here are few you may encounter frequently:

Folds in the section: Paraffin sections are heated to flatten them and make them adhere firmly to the slide. If they are not heated enough, folds in the tissue may occur. Cross sections of large tubular organs such as large blood vessels are particularly prone to folds. You can probably find some on slides of the iliac artery (Slide 31A or 31B) the renal artery (Slide 32C), or the pulmonary vein (Slide 35A). In blood vessels the folds tend to extend radially from the lumen. Students sometimes mistakenly think that they are ducts or small blood vessels emptying into the lumen of the larger vessel.

Tears: Tears often occur at weak points in the tissue, such as where one layer of an organ interfaces with another. Epithelia are especially likely to be torn off the surfaces that they line. An example would be in our slides of the larynx (Slide 45A or 45B), where the epithelium that covers the true vocal fold is often torn away from the underlying connective tissue. Another example is Slide 41A or 41B of the Plantar Skin (the skin on the soles of your feet). In this preparation, a large tear has developed in the connective tissue of the dermis (just below the dark-staining epithelium). It would be easy for a beginner to confuse this space with a large blood vessel or with the fatty tissue (adipose tissue) that lies deep to the dermis on this same slide.

V. ELECTRON MICROSCOPY

Lastly, study the ultrastructure of a typical cell using Rhodin’s atlas of electron micrographs (EMs). Observe the following:

1. Overview of typical organelles: Fig. 2-1. Identify the multiple stacks of Golgi cisternae, which are often clustered in a small area of cytoplasm (the cytocentrum or centrosome) near the nucleus. This is the area that often stains pale in H&E-stained sections for light microscopy (see Wheater Fig. 1.9c, p.15). A large cytocentrum indicates a very active Golgi, and is usually associated with cells that are secreting large amounts of proteins, or cells that are producing many lysosomes because they are highly phagocytic. The Golgi cisternae lack ribosomes, a feature that distinguishes them from the ribosome-studded cisternae of the rough endoplasmic reticulum (labeled “9” in Fig. 2-1). Within the nucleus, identify the heterochromatin vs. the euchromatin.

2. Plasmalemma (plasma membrane): Figs. 2-2 & 2-3. Notice that at very high magnification a single membrane has a trilaminar appearance (2 dark layers separated by a light layer). This is true of all membranes within a cell, not just the plasma membrane.
3. Nucleolus: Figs. 5-16, 2-80 & 2-84. Distinguish between the heterochromatin, the euchromatin, and the nucleolus in Figs. 5-16 & 2-80. At high magnification in favorable sections (Fig. 2-84) the filamentous (#4) & granular portions (#5) of the nucleolus can be resolved.

4. Identify the inner nuclear membrane, the outer nuclear membrane, the perinuclear cisterna & a nuclear pore at low magnification in Fig. 2-80 & at higher mag in Fig. 2-81.

5. Ribosomes, polysomes & rough endoplasmic reticulum: Figs. 2-27 to 2-30 & 7-29. Ribosomes form a grouping called a polysome when they are translating an mRNA strand. When viewed “en face” (French for “face on”) the ribosomes in a polysome often show a circular or spiral arrangement (Figs. 2-27 & 2-28), but the strand of mRNA is usually not visible in ordinary EMs. It is important to note that a polysome can be either free in the cytoplasm (Figs. 2-27 & 2-28) or fixed to the membrane of the rough endoplasmic reticulum (Figs. 2-29 & 2-30). Most cells have a mixture of free and fixed polysomes because different types of protein are made on each. Free polysomes tend to produce most of the proteins that will remain inside the cell for its own use. Fixed polysomes produce mostly secretory proteins, plus lysosomal enzymes and membrane proteins.

The rough endoplasmic reticulum or RER is organized into flattened sacs of membrane called cisternae, which have ribosomes in the form of fixed polysomes attached to their cytoplasmic surface (Fig. 2-30). Notice that when they are abundant, the cisternae of RER tend to be quite long, and tend to stack up in parallel rows (Figs. 7-27 & 2-29). Large amounts of RER usually mean that the cell is producing lots of secretory protein, since it only requires a small amount of RER to synthesize enough lysosomal enzymes and membrane proteins to supply most cells. Large amounts of RER would make the cytoplasm of a cell such as this one appear highly basophilic by light microscopy.

6. Smooth (agranular) endoplasmic reticulum (SER): Figs. 2-26 & 2-34. The SER is described as smooth or agranular because it lacks ribosomes on its surface. It tends to be organized into tortuous tubules rather than into sac-like cisternae as in the RER. In thin sections, an area rich in SER often looks as if it contains many small vesicles, which are really cross sections through the SER tubules. The arrow labeled “3” in Fig. 2-34 illustrates the fact that the SER tubules are physically continuous with the cisternae of the RER. Most cells have relatively little SER, making it very difficult to identify it with any certainty in ordinary EMs.

7. Golgi complex: Figs. 2-36 to 2-38. Note that Golgi cisternae are usually shorter and more curved than cisternae of RER (Fig. 2-37). Stacks of Golgi cisterna therefore typically have a convex surface called the forming face or cis face, and a concave surface called the maturing face or trans face. In many epithelial cells the Golgi is located between the nucleus and the free surface (apical surface) of the plasma membrane (Fig. 2-36). In this location it is often referred to as a supranuclear Golgi.

8. Mitochondria: Fig. 2-39 for the usual appearance; Figs. 2-40 to 2-43 for variants. Identify the outer membrane and the shelf-like cristae formed by folds of the inner membrane. The space that lies interior to the inner membrane is the matrix. Notice the unusual tubular cristae in Figs. 2-42 & 2-43. Tubular rather than shelf-like cristae are characteristic of mitochondria in most steroid-producing cells. Figs. 2-44 & 2-45 illustrate the fact that mitochondria replicate through fission.
9. **Lysosomes**: The morphology of lysosomes is very variable, and most cells contain several types. Since many of them look like ordinary vacuoles, positive identification often requires demonstration of lysosomal enzyme activity using enzyme histochemistry (see Ross, Fig. 2.23, p. 44). A few kinds of lysosomes have a distinctive and easily recognizable morphology. One example is an autophagic vacuole or autophagosome (Fig. 2-54 or Ross Fig. 2.23). In autophagy, one or more lysosomes surround a region of cytoplasm and then fuse to form a double-walled vacuole. Initially the lysosomal enzymes are restricted to the space between the two membranes of the vacuole (bracketed in the Rhodin figure). This space used to be the lumen of the lysosome. The inner membrane then breaks down, allowing the enzymes to gain access to the enclosed cytoplasmic region and digest it. Autophagy is one method used in the turnover of cytoplasmic organelles. Thus it is common to find polysomes, mitochondria, and other cytoplasmic components within an autophagosome. Lysosomes can sometimes be identified because they contain visible debris that they have not yet fully digested. An example is seen in Wheater, Fig. 1.13a, p. 19.

10. **Peroxisomes**: Peroxisomes have a granular matrix, which in some species (not in humans) contains a slightly darker crystalloid (Fig. 2-55). They can be distinguished from lysosomes histochemically.

11. **Lipid droplets**: Fig. 2-74. Note that despite what the caption of this figure implies, lipid droplets in the cytoplasm of a cell ARE NOT surrounded by a membrane. By EM, lipid droplets appear pale if the lipid has been extracted during fixation (Fig. 2-74), but are dark gray or black if the lipid has been preserved (Fig. 7-39).

12. **Secretory granules**: Fig. 7-25. Proteins destined for secretion bud off of the Golgi cisternae to form secretory vacuoles (secretory granules) that are surrounded by a membrane. Many cells store these secretory vacuoles in their cytoplasm until they receive a specific signal that tells them to secrete. This is called regulated secretion. Such cells tend to have numerous large vacuoles that usually contain highly concentrated, dark-staining secretory product. In contrast, other cell types secrete their products from the secretory vacuoles as soon as they are formed, without the need for any external signal. This is called constitutive secretion. The secretory granules of constitutive secretors tend to be small, pale-staining vesicles (Fig. 7-29) and are usually few in number since they are released quickly. (Note: Vesicles and vacuoles are both surrounded by a membrane. By convention a small structure is called a vesicle and a larger one is a vacuole).

13. **Microtubules**: Figs. 2-63, 2-67, & 2-73. Compare microtubules with intermediate filaments (Fig. 2-73). Which is hollow? (Answer: Microtubules.) Which has a larger diameter? (Answer: Microtubules). In Fig. 2-67 the microtubules are part of a mitotic spindle and are organized around a centriole.

IX. **VIDEODISK**

For this laboratory there are few videodisk images to study. It is sufficient to use the book of barcodes at each videodisk station to study examples of different stains as seen on pages 237 & 238. Look at the sections stained with: H&E, the various elastic fiber stains, reticular fiber stain, trichrome, PAS, Best’s glycogen stain, osmium, Masson stain, Sudan Black B, and Oil Red O.
### LABORATORY 1 CHECKLIST
**MICROSCOPY, STAINS & ORGANELLES**

#### LIGHT MICROSCOPY

<table>
<thead>
<tr>
<th>Feature</th>
<th>Stain/Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>smooth muscle in cross section</td>
<td>glycogen in Best's carmine stain</td>
</tr>
<tr>
<td>smooth muscle in longitudinal section</td>
<td>any PAS-positive structure</td>
</tr>
<tr>
<td>cytoplasm vs. nucleus vs. nucleolus</td>
<td>reticular fibers in a silver stain</td>
</tr>
<tr>
<td>Nissl body</td>
<td>elastic fibers stained with aldehyde-fuchsin</td>
</tr>
<tr>
<td>collagen in a trichrome stain</td>
<td>myelin stained with osmium</td>
</tr>
<tr>
<td>internal elastic lamina</td>
<td></td>
</tr>
</tbody>
</table>

#### ELECTRON MICROGRAPHS

<table>
<thead>
<tr>
<th>Structure</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Golgi cisternae</td>
<td>mitochondria</td>
</tr>
<tr>
<td>nucleus</td>
<td>lysosome</td>
</tr>
<tr>
<td>perinuclear cisterna</td>
<td>autophagic vacuole</td>
</tr>
<tr>
<td>nuclear pore</td>
<td>peroxisome</td>
</tr>
<tr>
<td>nucleolus</td>
<td>lipid droplet</td>
</tr>
<tr>
<td>heterochromatin</td>
<td>secretory granule (vacuole/vesicle)</td>
</tr>
<tr>
<td>euchromatin</td>
<td>microtubule</td>
</tr>
<tr>
<td>ribosomes</td>
<td>intermediate filament</td>
</tr>
<tr>
<td>free polysomes</td>
<td>centroli</td>
</tr>
<tr>
<td>rough endoplasmic reticulum</td>
<td></td>
</tr>
<tr>
<td>smooth endoplasmic reticulum</td>
<td></td>
</tr>
<tr>
<td>trilaminar appearance of any</td>
<td></td>
</tr>
<tr>
<td>membrane at high magnification</td>
<td></td>
</tr>
</tbody>
</table>

**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 1: MICROSCOPY, STAINS & ORGANELLES

Test your understanding of the material covered in this lab session by seeing whether you can answer the following questions. The correct answers are posted on the course website (http://neurobio.drexelmed.edu/education/ifm/microanatomy) under the heading “Lab Focus Questions”.

1. Which lens in a compound light microscope focuses the illumination from the light source onto the specimen?
2. Which is larger: a nanometer or a micron?
3. What is the diameter of an average red blood cell?
4. If you were cutting thin sections for electron microscopy, about how many serial sections would be required to section completely through a cell that was 20 microns in diameter? Would it be closer to ten sections, several hundred sections or several thousand?
5. Most trichrome stains were developed to stain _____________ & _____________ different colors.
6. Name a stain that you could use to stain elastic fibers.
7. Zenker’s and Bouin’s are names of common ______________ used in light microscopy.
8. Are cellular components that have a net negative charge in fixed tissues acidophilic or basophilic?
9. With H&E, do acidophilic structures tend to stain pink or bluish-purple?
10. What are Nissl bodies?
11. Why are Nissl bodies basophilic?
12. Why do the mitochondria of cardiac muscle cells contain many cristae?
13. Why do the mitochondria of the liver have a large matrix compartment? (Hint: What important metabolic processes occur within the matrix?)
14. What can you conclude about a cell that has many nucleoli or a single very large nucleolus?
15. Suppose a cell has a very basophilic cytoplasm except for one pale-staining region near the nucleus. What organelle would be abundant in the pale-staining region? In the basophilic region? What is the function of this cell likely to be?
LABORATORY 2
EPITHELIUM & GLANDS

I. EPITHELIUM

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

1. recognize and classify epithelial tissues, and describe their characteristics, functions, and locations

2. understand the functions of surface modifications including:
   - microvilli
   - stereocilia
   - cilia

3. give examples of the locations in which you would expect to find each of the major types of epithelium including:
   - simple squamous
   - simple cuboidal
   - simple columnar
   - stratified squamous (maximally keratinized and minimally keratinized varieties)
   - stratified cuboidal
   - stratified columnar
   - pseudostratified ciliated columnar
   - transitional

4. recognize the following by light microscopy in appropriately stained sections and understand their structure and function:
   - terminal web
   - terminal bar
   - basement membrane
   - brush border
   - goblet cells

5. recognize the following membrane junctions by electron microscopy and know their functions:
   - zonula occludens
   - zonula adherens
   - desmosome
   - gap junction (nexus)
   - hemidesmosome

LABORATORY:

During this laboratory session, you will be studying the various types of epithelia. Note that epithelia may develop from any of the three germ layers: ectoderm, mesoderm, endoderm. Epithelia cover body surfaces and line body cavities. While examining the slides, it is not necessary at this time to learn the detailed structure of each organ since they will be studied later during the portion of the course devoted to organ systems. For now, learn to locate and classify the epithelia in these organs, and understand their various functions, and structural specializations.
Epithelial classification is normally based on the number of cell layers in the epithelium and the shape of the cells in the surface layer. However, some epithelia have special names, e.g. endothelium and mesothelium. These names are based on the location of the epithelium rather than the number of layers or the shape of the cells.

A. SIMPLE EPITHELIA

Simple epithelia are one cell layer thick. They are further subdivided according to the shape of the cells into:

- simple squamous epithelium
- simple cuboidal epithelium
- simple columnar epithelium

Please study simple epithelia on the following slides in your set:

1. Simple Squamous Epithelium
   Slide 47 (HU Box): Artery and Vein, or
   Slide 35: Vena Cava
   and
   Slide 71 (HU Box): Lung, Human
   These slides contain several examples of simple squamous epithelia, including the endothelium, which lines all blood vessels (Slide 47HU or 35), and the mesothelium, which covers the outer surface of the lung (Slide 71HU). A squamous cell is much wider than it is tall. When viewed face on, it is often a polygonal cell with a centrally placed round or oval nucleus. When viewed from the side, simple squamous epithelial cells are flattened with a centrally located nucleus that may be thicker than the cytoplasm, creating a bulge similar to the yolk of a fried egg. The cytoplasm may be so thin that it is barely visible by light microscopy. You may only be able to see the basophilic nuclei. The thin cytoplasm allows for rapid transport across the epithelium.

2. Simple Cuboidal Epithelium
   Slide 74 (HU Box): Kidney, Human, or
   Slide 50: Kidney, Human or any kidney slide
   This slide, as well as many others in your box, contains examples of simple cuboidal epithelia. The height and width of cuboidal epithelial cells are approximately equal. Simple cuboidal epithelium lines the proximal tubules and distal tubules of the kidney. It is not necessary for you to distinguish between these tubule types at this point.
   On these same slides, you can also find two examples of simple squamous epithelium within the kidney. What are they? (Answer: The parietal layer of Bowman's capsule, and the capillary endothelium.)

3. Simple Columnar Epithelium
   Slide 60 (HU Box): Duodenum, c.s., or
   Slide 64: Jejunum
   and
   Slide 68 (HU Box): Liver and Gall Bladder, Monkey, or
   Slide 68E: Liver and Gall Bladder
   Columnar cells are distinguished by the fact that their height is greater than their width. Find the simple columnar epithelium that lines the lumen of the intestine and gall
bladder. Observe the membrane specializations on the apical surface of these cells and consult your atlas to find out what they are (Wheater, p. 93). What makes up the core of a cilium? (Answer: An axoneme composed of microtubules that are arranged in a 9+2 array.) What makes up the core of a microvillus? (Answer: A bundle of actin microfilaments and associated proteins.)

Some epithelia contain more than one mature cell type. Compare the gall bladder where all the epithelial cells look alike, with the intestine where two major cell types can be distinguished by LM - the goblet cell & the enterocyte or intestinal absorptive cell.

ARE YOU READY FOR A CHALLENGE? It is possible to section a simple epithelium in such a way that it appears to be stratified. Can you see how that could happen? See Fig. 6 on the following page for an explanatory diagram. Verify also that there is no way you can section a stratified epithelium and make it look like a simple epithelium.

B. STRATIFIED EPITHELIA

Stratified epithelia are by definition more than one cell layer thick. Some of the cells rest on the basement membrane, and some reach the free surface of the epithelium, but no individual cell does both. Stratified epithelia are further subdivided according to the shape of the cells at the FREE SURFACE of the epithelium. The shape of more basally located cells may be very different from that of the surface cells, but only the shape of the surface cells is relevant to the classification. Stratified epithelia are subdivided into:

- stratified squamous epithelium
- stratified cuboidal epithelium
- stratified columnar epithelium

1. Stratified Squamous Epithelium

   Slide 54 (HU Box): Skin, Palm or Sole, or Slide 41B: Plantar Skin
   and
   Slide 40: Skin, or Slide 42: Scalp
   Slide 56 (HU Box): Esophagus, c.s.

   The skin is an example of a maximally keratinized stratified squamous epithelium. It is characterized by a surface layer of heavily keratinized squamous cells that no longer contain nuclei. Compare the thick skin on slide 54 or 41B with the thin skin on slide 40 or 42. Regardless of its thickness, all skin has a heavily keratinized surface layer that helps to prevent desiccation.

   The esophagus is lined by minimally keratinized stratified squamous epithelium (also called nonkeratinized). In such epithelia at least some of the surface cells contain nuclei. This type of epithelium is generally found on moist internal surfaces of the body that are subjected to abrasion (such as the pharynx and vagina), while maximally keratinized stratified squamous epithelium is found on dry surfaces such as the skin.

   Note again the difference in cell shape in the surface layer vs. the basal layer of and recall that only the shape of the surface cells is considered when classifying the epithelium. The basal cells are the stem cells from which the cells in more superficial layers differentiate.
2. Stratified Cuboidal Epithelium  
   Slide 54 (HU Box): Skin, Palm or Sole, or  
   Slide 40 or 41: Skin  
   Stratified cuboidal epithelium is relatively rare but may be found in the ducts of sweat glands, and in parts of the duct systems of some other glands including salivary glands, and mammary glands. It usually involves two layers of cells.

3. Stratified Columnar Epithelium  
   Slide 84 (HU Box): Penis, Fetal, Masson Trichrome, or  
   Slide 85: Penis  
   Stratified columnar epithelium is quite rare. It is usually found only in small patches, usually where a stratified squamous epithelium changes into a pseudostratified columnar or simple columnar epithelium. In these cross sections of the penis, you may be able to find such patches of stratified columnar epithelium in the lining of the penile urethra. It is rarely more than two cells layers thick, and is characterized by a relatively neat, well-defined row of nuclei in each layer.

C. PSEUDOSTRATIFIED COLUMNAR EPITHELIUM

By definition a pseudostratified epithelium is one in which all cells are in contact with the basement membrane, and some (but not all) cells reach the free surface. The short basal cells are usually the stem cells that differentiate into the taller columnar cells. The connection of the columnar cells with the basement membrane is often via a thin strand of cytoplasm that may not be visible by light microscopy. Therefore pseudostratified epithelia often resemble truly stratified epithelia by light microscopy. (Sometimes the nuclei in a pseudostratified columnar epithelium are more randomly scattered rather than arranged in the neater rows that characterize truly stratified columnar epithelia, but this distinction is not always obvious). For now you should learn several examples of locations where pseudostratified columnar epithelium are known to be present in the body.
Many parts of the respiratory tract, including the trachea and the bronchi, are lined by a pseudostratified columnar epithelium with ciliated cells and goblet cells. It is also called "the respiratory epithelium" (even though other types of epithelium line the smaller airways). It can be immediately identified because no other epithelium has the combination of ciliated cells and goblet cells. Therefore when you see these two cell types together in an epithelium you know that you are looking at the respiratory epithelium and therefore at a pseudostratified epithelium.

The trachea is one of the few locations where the basement membrane is sometimes thick enough to be seen even in an H&E stained specimen. Check your slides to see if you can identify it.

D. TRANSITIONAL EPITHELIUM

This type of epithelium is called transitional because the shape of the surface cells changes as a result of distention of the organ. They tend to be rounded, dome-shaped cells in the empty organ, and more flattened rectangular cells in the distended organ. It is not uncommon to see both types of cells in different areas of the same section. Other characteristics of transitional epithelia include the fact that some of the surface cells may be binucleate, and that the surface cells are often larger than those in deeper layers of the epithelium.

E. MEMBRANE JUNCTIONS

Epithelial cells are usually connected to one another by several different types of membrane junctions. Know the structure of the various types of junctions as seen by LM, thin section TEM and freeze fracture. Observe the following slides to see what some of these junctions look like when they are visible by light microscopy, and then study their appearance by TEM.

Slide 64: Jejunum

Look carefully and see if you can locate a terminal bar. It is a very small dark dot on the lateral plasma membranes near the apical ends of the cells. It includes all the junctions that make up a junctional complex - zonula occludens, zonula adherens, and desmosomes. These cells also have abundant microvilli on their apical surfaces, making up the striated border. The cores of the microvilli anchor in the terminal web located in the clear area of cytoplasm just beneath the apical plasma membrane.
Slide 54 (HU Box): Skin, Palm or Sole, or: 
Slides 40 or 41B: Skin

Use your atlas to identify the stratum spinosum of the skin. Locate this layer on the slide and see if you can identify the small spines between epithelial cells in this layer. What type of membrane junction does each spine represent? (Answer: Desmosome).

F. ELECTRON MICROSCOPY (RHODIN)

Good examples of different epithelial types are found in the following micrographs:

1. Simple squamous (Figs. 3-3 & 3-4): The height of the cell is much less than the width. There are relatively few organelles. The thinness of the cytoplasm allows rapid transport across the epithelium.

2. Simple cuboidal (Fig. 3-7): The height of the cell is approximately equal to the width. The nucleus is usually round. Find the lateral boundaries between the cells (#3 in the figure, where they are erroneously labeled “cell bodies” instead of “cell boundaries”). Follow the lateral cell boundaries toward the apical end of the cells and identify the dark-staining junctional complex. Junctional complexes can occur in many other epithelial types in addition to simple cuboidal. At this low mag it is not possible to distinguish the 3 different membrane junctions that make up each junctional complex (the zonula occludens, zonula adherens & desmosome – see below).

3. Simple columnar (Figs. 3-8 to 3-10): The height of the cells is greater than the width. The nucleus tends to be in the basal half of the cell, and is often oval rather than round. Columnar and cuboidal cells are more likely to have specializations of the apical plasma membrane (microvilli, cilia or stereocilia) than are squamous cells.

4. Stratified squamous: Figs. 3-12 & 3-13 show minimally keratinized (nonkeratinized) stratified squamous epithelium, while Figs. 3-14 & 3-15 show maximally keratinized stratified squamous epithelium. In the minimally keratinized variety, the surface cells are still viable and many still contain nuclei (Fig. 3-12). In contrast, the surface cells of the maximally keratinized variety are dead and lacking nuclei (anucleate) and represent little more than bags of keratin.

5. Pseudostratified columnar (Figs. 3-11, 31-21 & 31-22): In some pseudostratified epithelia there are several different types of columnar cells, all of which have differentiated from the same population of basal cells. The respiratory epithelium seen in these micrographs contains two types of columnar cells: ciliated cells and goblet cells. Also identify the small round basal cells (#6).

6. Transitional (Figs. 3-20, 32-34 & 32-35): Note that the surface cells are larger than those in the lower layers. The surface cells contain what appear to be flat vesicles, here labeled discoid vesicles that may actually be invaginations of the plasma membrane. These are shown at higher mag in Figs. 32-34 and 32-35. They are apparently important in rapidly increasing the surface area of the plasma membrane when the bladder is distended.
Membrane Specializations:

1. **Cilia**: Even at low magnification cilia can be identified because they grow from basal bodies in the cytoplasm (Fig. 3-10 & 2-21). Study the structure of cilia at higher magnification (Figs. 2-22 to 2-25). Notice that the main portion of the cilium (Fig. 2-22C) has a 9+2 arrangement of microtubules (9 doublets surrounding 2 individual central microtubules) while the basal body (Fig. 2-22E) consists only of 9 microtubule triplets, just as in a centriole (Fig. 2-61). Cilia are found on some, but not all, columnar and cuboidal epithelial cells. Cilia are most common on columnar and cuboidal cells.

2. **Stereocilia** (Figs. 2-16, 2-17 & 3-11): Stereocilia are not cilia at all, because they contain no microtubules. They are actually long, branching microvilli complete with cores of actin filaments (Fig. 2-16). They are found in relatively few organs (e.g., the epididymis in the male reproductive tract). They were named by light microscopists who, aware of the fact that cilia are usually longer than microvilli, assumed incorrectly that these extremely long structures must be cilia.

3. **Microvilli** (Figs. 3-9, 32-13 & 32-14): When a cell has many closely packed microvilli at its apical end as in all these micrographs, they form a layer called a brush border or striated border, which may even be visible by LM in favorable preparations. Many other cell types have small numbers of short microvilli visible only by EM (Figs. 2-19, 3-6 & 3-8).

**Glycocalyx** (Figs. 2-2 & 2-18): A thin glycocalyx is associated with the extracellular surface of the plasma membrane in many cell types, but is particularly well-developed on the microvilli of the enterocytes (absorptive cells) of the intestine. A glycocalyx consists of membrane lipids & membrane glycoproteins that extend into the extracellular space. In the intestine it includes enzymes important in the digestive and absorptive functions of these cells.

Find the bundle of actin microfilaments that forms the core of each microvillus, and note that they are anchored in the terminal web at the apical end of the cell (Fig. 2-18).

Membrane junctions:

1. **Zonula occludens** (tight junction) (Figs. 2-4 & 2-5): The 2 membranes are very close together. At higher mag than these micrographs, the membranes appear to fuse at multiple points and then diverge slightly between the fused regions. Each fusion point is felt to represent the location of one of the sealing strands that are visible by SEM. A zonula occludens is always part of a junctional complex.

2. **Zonula adherens** (Figs. 2-4 & 2-5): The two membranes are not fused. Presumably the cadherins extending from the membranes of neighboring cells interact in the space between the membranes, but they are not visible by EM. The cytoplasmic plaque is the attachment site for actin microfilaments. A zonula adherens is always part of a junctional complex.

3. **Desmosome** (Figs. 2-5 & 2-9): The membranes are separated by an intercellular space that often contains a dense line (#5, Fig. 2-9) thought to be formed by interacting desmogleins and desmocollins. The cytoplasmic plaque (#6, Fig. 2-9) is darker than in a zonula adherens. It serves as an attachment site for intermediate filaments. Desmosomes may be found as part of a junctional complex, or they may
occur elsewhere over the surface of cell membrane, unaccompanied by a zonula occludens or zonula adherens.

4. **Hemidesmosome** (Fig. 2-10): Resembles half of a desmosome. Attaches the cell not to a neighboring cell but rather to the basal lamina on which the epithelial layer rests (torn away in this micrograph). Therefore in a stratified epithelium hemidesmosomes are only found on the basal cells. Hemidesmosomes attach to the basal lamina via integrins.

5. **Gap junction** (Fig. 2-6): This is a poor micrograph of a gap junction. A better one is in Wheater, Fig. 5.12a, p. 91. Gap junctions are best identified by the facts that:
   - they are never part of a junctional complex
   - the membranes are not fused but are closer to one another than in a zonula adherens or desmosome
   - there is no cytoplasmic plaque associated with a gap junction since no cytoskeletal filaments anchor to them
   - the membranes are strictly parallel to one another (no fusing and diverging as in a zonula occludens)

II. **GLANDS**

**OBJECTIVES:**
At the end of this unit, you should be able to:

1. describe the differences between exocrine and endocrine glands in terms of their development and structure
2. define & give examples of merocrine, apocrine & holocrine secretion
3. understand the classification system for exocrine glands based on the branching pattern of the duct and the shape of the secretory portion
4. recognize the following types of ducts:
   - intralobular ducts including:
     - intercalated ducts
     - striated ducts
   - interlobular ducts
   - main or excretory ducts
5. recognize, classify and become familiar with the structure of:
   - salivary glands
   - sweat glands
   - sebaceous glands
   - myoepithelial cells
   - serous demilunes

**INTRODUCTION:**

Glands can be classified in many ways, each based on a different aspect of their structure or function. For example they can be divided into:

- exocrine vs. endocrine based on whether they secrete onto a free surface of the body (exocrine) or into the bloodstream (endocrine)
- merocrine, holocrine or apocrine based on how they release their secretory product
- unicellular vs. multicellular
- intraepithelial, intramural, or extramural based on the location of the gland

More specialized distinctions apply to certain groups of glands. For example:
- protein-producing glands are either mucous or serous depending on whether they produce a thick viscous secretion (mucous glands) or a thin watery secretion (serous glands)
- exocrine glands with ducts are either simple or compound depending on whether the duct branches (compound glands) or does not branch (simple glands)
- multicellular exocrine glands are often divided into tubular, acinar (alveolar) or tubuloacinar (tubuloalveolar) glands based on the shape of the secretory unit.

Glands that secrete their product onto a free surface of the body are termed exocrine glands. They are usually connected to that surface by way of a duct. In contrast, endocrine glands are ductless and secrete their products (hormones) into the surrounding connective tissues and thence into the bloodstream. (Note that we will not study the detailed structure of endocrine glands now, since there is a separate unit on these organs later in the course.)

Glands can be classified as merocrine, apocrine, or holocrine based on how the secretory product is released from the cell (mode of secretion) (Fig. 7). In merocrine secretion the product is released by exocytosis (fusion of the membrane of the secretory vacuole with the plasmalemma) and no part of the cytoplasm other than the secretory product itself is lost. Most glands use merocrine secretion. In apocrine secretion, a portion of the cell is lost along with the secretory product. What usually happens is that the secretory material, surrounded by a thin rim of cytoplasm and by plasma membrane, buds off from the cell. Thus, in addition to the secretory product, the cell loses the rim of cytoplasm and the part of the plasma membrane surrounding it. The mammary gland secretes milk lipids in an apocrine fashion. In holocrine secretion the entire secretory cell is shed and lost during the process of secretion. It may remain intact, in which case the cell itself is the secretory product (e.g., sperm), or it may disintegrate to release its secretory products (e.g., sebaceous glands).

![Fig. 7: The Three Modes of Secretion](image-url)
Glands can be classified as either unicellular or multicellular. A good example of a unicellular exocrine gland is the goblet cell of the intestines and respiratory tract. The enteroendocrine cells of the gut are unicellular endocrine glands. Most exocrine and endocrine glands are multicellular.

Glands can be classified according to their location as intraepithelial, intramural, or extramural. Intraepithelial glands are located within an epithelium; i.e., no part of the gland extends beyond the epithelium. Intraepithelial glands may be unicellular (e.g., the goblet cell or enteroendocrine cells) or they may be multicellular sheets (e.g., the surface cells lining the lumen of the stomach, which all secrete mucus). Intramural glands are located within the wall of an organ (murus is the Latin word for wall), but they extend beyond the epithelium into other layers of the wall such as the underlying connective tissue. An example would be Brunner’s glands, which are located within the wall of the duodenum, but in the submucosal layer of connective tissue rather than in the epithelium. Extramural glands are located outside the wall of an organ and are connected to the organ only by a duct. Extramural glands include the liver, gall bladder and pancreas, which lie outside the wall of the duodenum but are connected to it by ducts.

Protein-secreting cells may be classified as either mucous or serous. In addition to a difference in the viscosity of their secretions, there are differences in morphology between the two types of the cells. Mucous cells store their product in secretory vacuoles that often compress the nucleus against the basal plasma membrane. Also, the contents of the secretory vacuoles are easily extracted during routine fixation, and hence the apical region of the cell is usually pale-staining. In contrast, the secretory vacuoles of serous cells are more resistant to extraction, and the proteins that they contain cause the apical cytoplasm to be acidophilic. In addition, their nucleus is more likely to remain round rather than becoming flattened. Based on the abundance of serous vs. mucous cells, a gland can be classified as a pure mucous gland, a pure serous gland, or a mucoserous gland.

The final classification scheme is an old one that is now infrequently used. It applies only to multicellular exocrine glands with ducts. These can be further subdivided based on whether or not the duct branches, and the shape of the secretory portions:

If the duct does not branch, the gland is a simple gland; if the duct branches it is a compound gland.

If the secretory portions are shaped like a tube, the gland is termed tubular. If they are spherical it is an acinar or alveolar gland. If both tubular and acinar secretory portions are present, it is a tubuloacinar or tubuloalveolar gland. Mucous secretory portions tend to be tubular, while serous secretory portions tend to be acinar. Tubuloacinar glands therefore are usually mucoserous glands.

The following rules should clarify this nomenclature:

1. The first word in the official description of the gland describes the duct. It is either “simple” or “compound”, depending on whether the duct is unbranched or branched.

2. The rest of the description applies to the secretory portion of the gland. If the secretory portion branches or coils, then the term "branched" or "coiled" is used as the second word in the description. Thus a simple branched gland has an unbranched duct (simple) and a branched secretory portion.
3. The final word in the classification describes the shape of the typical secretory portion: tubular, acinar (alveolar), or tubuloacinarian (tubuloalveolar).

This classification scheme is still used when describing a few glands such as the intestinal glands (simple tubular), eccrine sweat glands (simple, coiled tubular), the exocrine part of the pancreas (compound acinar), or the submandibular salivary gland (compound tubuloacinarian). Many other exocrine glands do not fit into this classification scheme.

LABORATORY:

Please study the following slides in your set:

A. SALIVARY GLANDS
   Slide 12 (HU Box): Parotid Gland, or
   Slide 57A: Parotid Gland
   and
   Slide 13 (HU Box): Salivary Gland, Sublingual, Human, or
   Slide 57B: Sublingual Gland
   and
   Slide 14 (HU Box): Stratified Columnar Epithelium, Human (Submandibular Gland), or
   Slide 57C: Submandibular Gland

   Study the three major salivary glands. Identify the cell types making up the secretory units and determine whether each gland is pure serous, pure mucous or a mixed mucoserous gland. (Answer: Parotid = pure serous, sublingual = mostly mucous, submandibular = more serous than mucous). In the sublingual gland, find a serous demilune. The cells of the serous demilune secrete into the lumen of the gland via small channels between the mucous cells.

   The salivary glands are large compound glands. Such glands are often organized into lobules and lobes. Identify a lobule. This is a group of secretory units packed close together, and separated from other lobules by septa (sheets) of connective tissue.

   Study the organization of the duct system of each lobule. Locate an example of an intralobular duct and an interlobular duct. The major criterion that distinguishes them is that intralobular ducts are within a lobule (Latin, intra = within) and are therefore directly surrounded by secretory units; interlobular ducts are in the septa between lobules (Latin, inter = between) and are therefore directly surrounded by connective tissue. Intralobular ducts drain into interlobular ducts, and hence the latter are usually larger.

   Salivary glands have two specialized types of intralobular ducts: intercalated ducts and striated ducts. They can be distinguished by the type of epithelium that lines them. Intercalated ducts have a simple, low cuboidal epithelium, while striated ducts have a simple cuboidal epithelium with eosinophilic stripes or striations in the basal region of the cytoplasm. These striations are caused by numerous mitochondria that line up in rows located between deep infoldings of the basal plasma membrane. The increased membrane surface area produced by the folding and the large number of mitochondria located nearby are classic features of cells that are heavily engaged in active transport across the plasmalemma. The increased surface are allows for many
more copies of the transporter molecules, and the mitochondria provide the ATP to drive the active transport.

In many compound glands a single duct eventually leaves each lobule. Several of these merge to form a lobar duct. All the lobules that drain into the same lobar duct make up a lobe, and each gland has multiple lobes that eventually drain into the main or excretory duct. Usually you cannot identify a lobe in a single section since you would need serial sections to trace the connections of the ducts. Lobar ducts eventually unite to form the main or excretory duct. You will probably not see lobar or excretory ducts in your sections because they are located at some distance from the secretory units. They would be surrounded by connective tissue, would be very large, and would be more likely to be lined by stratified epithelia as they increased in size.

B. **ECCRINE SWEAT GLANDS**
   Slide 54 (HU Box): Skin, Palm or Sole, or Slide 41: Plantar Skin
   Identify the eccrine sweat glands and their ducts. Classify these glands (simple or compound, tubular or acinar, straight or coiled, etc.). *(Answer: They are simple, coiled tubular glands.)* Distinguish the secretory portions of these glands from the ducts. The secretory portion tends to be paler staining and wider in diameter. It is surrounded by myoepithelial cells while the duct is not. The bright eosinophilic cytoplasmic processes of the myoepithelial cells can usually be identified around the periphery of the secretory portion. The duct is lined by a 2-layered stratified cuboidal epithelium.

C. **SEBACEOUS GLANDS**
   Slide 53 (HU Box): Lip, Skin (Thin), or Slide 44: Eyelid
   In the skin of the lip (Slide 53HU), locate the sebaceous glands. As in most locations in the body, the sebaceous glands on the outer surface of the lip are associated with hair follicles. The glands on the red portion of the lip (the vermillion border) are not. On this slide, make sure that you are looking at the sebaceous glands and not at the salivary glands that are located deeper in the connective tissue of the lip. Sebaceous glands secrete in a holocrine fashion. Note that the undifferentiated cells are located peripherally, resting on the basement membrane of the gland epithelium. As they mature they move toward the center of the gland and toward the very short duct. The more mature cells develop small, dark irregularly shaped nuclei (pyknotic nuclei) that are typical of cells about to die by the process of necrosis, which involves fragmentation of the nuclear and cytoplasmic membranes. The cytoplasm of the differentiated cells contains many lipid droplets that are usually lost during fixation, making the cytoplasm pale-staining. The eyelid (Slide 44) contains a type of modified sebaceous gland called a Meibomian gland. These extremely large glands are not associated with hair follicles.

D. **INTRAEPITHELIAL GLANDS**
   Slide 16 (HU Box): Stomach and Duodenum, l.s., or Slide 62A: Pyloroduodenal Junction
   Identify the surface mucous cells of the stomach, and the goblet cells of the duodenum. The surface mucous cells are an example of a multicellular sheet of secretory cells, while each individual goblet cell is a unicellular gland. Both are examples of intraepithelial glands.
E. ELECTRON MICROSCOPY (RHODIN)

Study the morphology of the following gland cell types:

1. Goblet cells (Fig. 4-1): Note the tapering basal half of the cell which resembles the stem of a goblet, the nucleus which is usually compressed in the stem region of the goblet, the secretory vacuoles containing mucus at the apical end of the cell, and the pale-staining region between the nucleus and the secretory vacuoles where the multiple stacks of Golgi cisternae would be located. This is referred to as the supranuclear location of the Golgi, and is typical of many epithelial cells.

2. Mucous cells of the salivary glands (Figs. 4-10 & 27-9): Compare these cells, which are pyramidal in shape, with the distinctive shape of goblet cells. Both cell types produce mucus, but their morphology is quite different. Note that in the salivary glands the nuclei of the mucous cells tend to become flattened at the base of the cell as large numbers of mucus-containing vacuoles accumulate in the cytoplasm. Mucus is a highly water-soluble mixture of glycoproteins and is often extracted by the aqueous solutions used in tissue processing. At low magnification they resemble lipid droplets whose contents have been extracted by organic solvents. At high mag the two structures could be distinguished because the secretory vacuole would be surrounded by a membrane and the lipid droplet would not.

3. Serous cells: Serous cells have a more rounded nucleus than mucous cells and the contents of their secretory granules are usually better preserved (darker-staining). In different glands the serous cells may be present as serous acini (Fig. 27-3), serous demilunes (Figs. 4-9 & 4-10) or in both forms.
# LABORATORY 2 CHECKLIST
## EPITHELIA & GLANDS

### LIGHT MICROSCOPY

<table>
<thead>
<tr>
<th>Simple Squamous Epithelium</th>
<th>Spines of the Stratum Spinosum (Desmosomes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelium</td>
<td>Serous Gland Cells</td>
</tr>
<tr>
<td>Mesothelium</td>
<td>Mucous Gland Cells</td>
</tr>
<tr>
<td>Simple Cuboidal Epithelium</td>
<td>Mixed Mucoserous Gland</td>
</tr>
<tr>
<td>Simple Columnar Epithelium</td>
<td>Serous Demilune</td>
</tr>
<tr>
<td>Goblet Cell</td>
<td>Parotid Gland</td>
</tr>
<tr>
<td>Enterocyte</td>
<td>Sublingual Gland</td>
</tr>
<tr>
<td>Maximally Keratinized Stratified Squamous Epithelium</td>
<td>Submandibular Gland</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Minimally Keratinized Stratified Squamous Epithelium</th>
<th>Keratinized Stratified Squamous Epithelium</th>
<th>Intralobular Duct (Including Intercalated &amp; Striated Varieties)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stratified Cuboidal Epithelium</td>
<td></td>
<td>Interlobular Duct</td>
</tr>
<tr>
<td>Stratified Columnar Epithelium</td>
<td>ECCRINE SWEAT GLAND (SECRETORY PART VS. DUCT)</td>
<td></td>
</tr>
<tr>
<td>The Respiratory Epithelium</td>
<td>Myoepithelial Cell</td>
<td></td>
</tr>
<tr>
<td>Transitional Epithelium</td>
<td>Sebaceous Gland</td>
<td></td>
</tr>
<tr>
<td>Terminal Bar</td>
<td>Meibomian Gland</td>
<td></td>
</tr>
<tr>
<td>Striated (Brush) Border</td>
<td>Surface Mucous Cells of Stomach</td>
<td></td>
</tr>
</tbody>
</table>

### ELECTRON MICROGRAPHS

<table>
<thead>
<tr>
<th>Simple Squamous Epithelium</th>
<th>Ciliated Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple Cuboidal Epithelium</td>
<td>Basal Bodies</td>
</tr>
<tr>
<td>Simple Columnar Epithelium</td>
<td>Goblet Cells</td>
</tr>
<tr>
<td>Junctional Complex</td>
<td>Transitional Epithelium</td>
</tr>
<tr>
<td>Zonula Occludens</td>
<td>Cilia</td>
</tr>
<tr>
<td>Zonula Adherens</td>
<td>Stereocilia</td>
</tr>
<tr>
<td>Desmosome</td>
<td>Microvilli</td>
</tr>
<tr>
<td>Hemidesmosome</td>
<td>Terminal Web</td>
</tr>
<tr>
<td>Gap Junction</td>
<td>Striated (Brush) Border</td>
</tr>
<tr>
<td>The Respiratory Epithelium</td>
<td>Glycocalyx</td>
</tr>
</tbody>
</table>

**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 2: EPITHELIA & GLANDS

Test your understanding of the material covered in this lab session by seeing whether you can answer the following questions. The correct answers are posted on the course website (http://neurobio.drexelmed.edu/education/ifm/microanatomy) under the heading “Lab Focus Questions”.

1. Define what is meant by the term “endothelium”.
2. Define what is meant by the term “mesothelium”.
3. Epithelia are avascular. How do the cells receive nutrients?
4. What is the function of cilia?
5. What is the function of microvilli?
6. Are cilia present on all epithelial cells? Are microvilli?
7. Which membrane junctions are specialized for mechanical adhesion? How do these junctions differ from one another?
8. Which membrane junction is specialized for cell-cell communication?
9. Which membrane junction acts as a permeability barrier to restrict the passage of molecules from one side of an epithelial sheet to the other?
10. In a pseudostratified or a stratified epithelium such as seen in the diagram below, where would you be most likely to find a zonula adherens: Only at location “A” (between surface cells), only at location “B” (between a basal cell and the basement membrane), or between any of the cells in any of the layers? Where would a zonula adherens be found? A desmosome? A hemidesmosome? A gap junction?

11. What is the relationship between an epithelium and a basement membrane?
12. What is the difference between a basement membrane and a basal lamina?
13. What is a terminal web? Where is it found? What cytoskeletal elements make up a terminal web?
14. Would serous demilunes be present in a pure serous gland?
15. In a striated duct, what causes the eosinophilic stripes or striations at the basal end of the epithelial cells?
16. What does the presence of basal striations tell you about the main function of striated duct cells?
17. Do all exocrine glands have striated ducts?
LABORATORY 3

CONNECTIVE TISSUE PROPER & ADIPOSE TISSUE

OBJECTIVES:

At the end of this lab, you should:

1. be familiar with the three major constituents of connective tissues:
   - cells
   - extracellular fibers
   - ground substance

2. recognize by light microscopy (on the videodisks and if possible also on glass slides) the major cell types found in connective tissue:
   - fibroblasts
   - macrophages
   - mast cells
   - adipocytes
   - lymphocytes
   - plasma cells
   - eosinophils
   - neutrophils
   - basophils

3. recognize by electron microscopy the following cell types:
   - fibroblasts
   - macrophages
   - mast cells
   - adipocytes
   - lymphocytes
   - plasma cells
   - eosinophils

4. recognize by light and electron microscopy the three major types of extracellular fibers found in connective tissue:
   - collagen fibers
   - elastic fibers
   - reticular fibers

5. understand the classification of connective tissue and identify by light microscopy the following types of connective tissue:
   - loose (areolar) connective tissue
   - dense irregular connective tissue
   - dense regular connective tissue
   - reticular connective tissue
   - mucous connective tissue
   - adipose tissue

6. be able to give examples of locations where each of these types of connective tissue is normally found

7. distinguish between brown fat and white fat
LABORATORY:

Connective tissues (CTs) perform many functions. Almost all epithelia are supported by a CT layer. CTs are the site of most inflammatory responses and many immune responses. They connect muscle to bone as tendons, and bone to bone as ligaments. They fill the spaces between organs, form the capsules and sheaths that surround organs, and provide internal support for the cells comprising most organs. CT forms the stroma or supporting elements of most organs. (The other tissues that carry out the unique functions of the organ are referred to as the parenchyma of the organ).

Connective tissue is one of the four basic tissue types. It can be distinguished from the others (epithelia, muscle and nerve) because:

1. CT cells are usually widely separated from one another by an extensive extracellular matrix that they have produced, rather than being in close proximity to one another.
2. most CTs contain a greater variety of differentiated cell types than found in other tissues (i.e., CTs usually have a highly mixed cell population).

There are 2 major groups of CT cells. One is the fibroblast family, which includes ordinary fibroblasts, reticular CT cells, adipocytes, osteoblasts in bone, chondroblasts in cartilage, etc.). In most CTs it is the members of the fibroblast family that produce most of the components of the extracellular matrix. Cells of the fibroblast family differentiate from the mesenchymal cells of embryonic CT.

The second group of CT cells is the bone marrow-derived cells, which arise from stem cells in the bone marrow and then migrate into CT to function there. These cells include monocytes (and macrophages derived from them), lymphocytes (and plasma cells derived from them), neutrophils, eosinophils, basophils and mast cells.

Another way of classifying CT cells is to divide them into resident cells (also called “fixed” cells) and wandering cells (also called “immigrant”, “transient”, or “elicited” cells). Resident cells are those that are usually found in relatively constant numbers in a particular CT (fibroblasts, adipocytes, mast cells, mesenchymal cells, macrophages), while wandering cells can migrate into a CT in large numbers in response to specific stimuli (lymphocytes, plasma cells, neutrophils, eosinophils, basophils, monocytes).

The extracellular matrix includes fibers and ground substance. There are 3 main fiber types: collagen fibers, reticular fibers and elastic fibers. The ground substance includes all the non-fibrous components of the matrix such as glycosaminoglycans (GAGs), proteoglycans, and various adhesive glycoproteins (e.g., fibronectin).

Combining the various cell types, fiber types and ground substance components in different proportions can produce CTs with very different properties and functions. In this lab we will study the following CT types:

**CT proper:**
- Loose (areolar) CT
- Loose cellular CT (the lamina propria of the gut)
- Dense regular CT
- Dense irregular CT

**Specialized CTs:**
- Reticular CT
- Adipose tissue
- Mucous CT

Other specialized CTs such as cartilage, bone, blood and bone marrow will be topics of separate labs.
I. CONNECTIVE TISSUE PROPER

A. Loose (Areolar) Connective Tissue
   Slide 20 (HU Box): Mammary Gland, Inactive, or
   Slide 34A or B: Mesentery
   Loose CT is characterized by relatively small numbers of fibers, a large amount of
   ground substance and a greater variety of cell types than other kinds of CT. The
   fibroblast is the most common cell type, but leukocytes, adipocytes, mast cells and
   macrophages are also present. The fibers tend to be thin and randomly oriented, and
   are mainly collagen fibers. Loose CTs bind other tissues together, and surround blood
   vessels, nerves, hair follicles, etc. The gel-like nature of the ground substance aids
   diffusion through the tissue. In these slides look for the loose CT around blood or
   lymphatic vessels (Slide 34A or B) and within the lobules of a gland (Slide 20 HU).

   Slide 62 (HU Box): Ileum, or
   Slide 64: Jejunum
   A loose CT layer called the lamina propria is part of the wall of many hollow
   organs (e.g., GI tract). It lies just beneath the epithelium that lines the lumenal surface of
   these organs. The epithelium and lamina propria are part of a layer called the mucosa.
   Lamina propria is a true loose CT. However, throughout most of the GI tract it is so
   highly cellular that it is almost impossible to find the fibers amid the large number of
   cells. The lamina propria in such locations is sometimes given a special designation and
   is called loose cellular connective tissue. Confirm for yourself on these slides the large
   number of cells in the lamina propria. Many of these cells are leukocytes, especially
   lymphocytes.

B. Dense Regular Connective Tissue
   Slide 97 (HU Box): Tendon, c.s. & l.s.
   or
   Slide 2: Tendon, l.s., and
   Slide 3: Tendon, c.s.
   A dense regular connective tissue is one in which there are many thick, closely
   packed fibers which are regularly (i.e., non-randomly) arranged. The fiber type is usually
   predominantly collagen fibers. There are fewer cells than in loose CT and almost all are
   fibroblasts. Note the different appearance of the fibroblast nucleus when cut in cross
   section (round appearance) vs. longitudinal section (elongated or oval appearance).
   Also notice that in a tendon the collagen fibers are all arranged in the same direction
   (parallel to the long axis of the tendon), thus providing excellent resistance to stretching
   forces that are consistently applied in the same direction. In a few dense regular CTs
   the arrangement of fibers is more complex. For example, the cornea resembles plywood
   in that the collagen fibers within each individual layer of corneal stroma are all oriented
   parallel to one another, but neighboring layers are arranged so that the fibers are at an
   angle to one another. Despite these differences in fiber orientation, both cornea and
   tendon are classified as dense regular CT because the direction of their collagen fibers
   is predictable (non-random).

C. Dense Irregular Connective Tissue
   Slide 54 (HU Box): Skin, Palm or Sole, or
   Slide 41: Plantar Skin (Aldehyde Fuchsin-Masson Trichrome)
In a dense irregular CT: (1) there are more collagen fibers than cells or ground substance; (2) the majority of cells are fibroblasts; and (3) the fibers are not arranged in any regular or predictable orientation. Dense irregular CT is well suited to resisting strong forces that may be applied from any direction. It is found in locations such as the dermis of the skin.

The dermis is the CT layer immediately beneath the epithelium. It has 2 indistinctly separated layers, a narrow papillary layer and a wider reticular layer, each composed of a different type of connective tissue. Consult an atlas to locate these layers. The papillary layer contains loose CT, while the reticular layer contains dense irregular connective tissue.

Slide 41 has been stained with aldehyde fuchsin & Masson trichrome. Masson trichrome stains collagen aqua (greenish blue). In some versions of this slide some of the larger collagen fiber bundles are red. This represents a staining artifact indicating that, in one of the steps of the staining procedure, the sections were not rinsed long enough to remove the red stain from all the collagen fibers. Note that although collagen fibers predominate, there are other fiber types present in both layers of the dermis. Find the elastic fibers, which are stained purple by the aldehyde fuchsin stain. Reticular fibers are also present but are not identifiable with this stain.

II. ELASTIC & RETICULAR FIBERS

A. Elastic Fibers

Slide 46 (HU Box): Aorta, Human, Elastic Stain, or Slide 29C: Aorta (Aldehyde Fuchsin) and Slide 72 (HU Box): Lung, Elastic Stain, or Slide 48B: Lung, (Elastic Fibers)

Identify the elastic fibers in the aorta and lung. Elastic fibers stain very poorly with H&E, and therefore usually require specialized stains (e.g., aldehyde fuchsin, orcein) to be visualized clearly. We have already seen in the dermis of the skin and again in slide 29C that aldehyde fuchsin stains elastic fibers purple. Other elastic stains cause them to stain brown, black, red, yellow or orange. Therefore you must know the stain used, or be familiar with the normal abundance and location of elastic fibers in different organs in order to identify them confidently. In elastic arteries such as the aorta the elastic tissue forms elastic sheets (elastic lamellae) rather than individual fibers. These sheets are concentrically arranged in the smooth muscle layer of the vessel called the tunica media (see Ross, Fig. 13.17a, p.419).

In the lung, the elastic fibers are less obvious. They are present in the walls of blood vessels and large airways, but also in the walls of the alveoli where gas exchange occurs. Excessive destruction of these elastic fibers seems to be involved in some forms of emphysema.

In most CTs elastic material is a minor component of the matrix. However in the tunica media of elastic arteries and in some elastic ligaments (ligamenta flava of the vertebral column, ligamentum nuchae of the neck, vocal cord of the larynx) it is so abundant that these tissues are sometimes referred to as elastic connective tissues.

B. Reticular Fibers

Slide 51 (HU Box): Spleen or Lymph Node (depending on your box) - Silver, (Reticular Fibers)
and

Slide 26B: Lymph Node, Reticular Fiber, or
Slide 28A: Spleen, Reticular Fiber

Identify the reticular fibers. Reticular fibers tend to form a meshwork or “reticulum” of fibers rather than thick fiber bundles. They provide a delicate supporting network for many soft organs such as the liver, the spleen, adipose tissue, lymph nodes, and many endocrine organs. They are also found underlying the basal lamina of epithelia. What is the chemical composition of reticular fibers? (Answer: They are composed of collagen type III).

In ordinary H&E preparations it is usually impossible to distinguish reticular fibers from other types of collagen fibers. However, after appropriate pretreatment of the sections, certain silver stains will stain reticular fibers black. Note that some of our slides have also been counterstained with a red dye that stains cell nuclei and cytoplasm in order to show the location of the cells relative to the fibers.

Reticular fibers are also PAS positive because they are highly glycosylated. Other types of collagen are less heavily glycosylated and hence are PAS negative. Since elastic fibers and other types of collagen fibers do not stain with silver stains or with the PAS technique, either of these procedures can be used to unequivocally identify reticular fibers.

III. SPECIALIZED CONNECTIVE TISSUES
A. Adipose Tissue

Slide 44 (HU Box): Cardiac Muscle, or
Slide 36: Left Ventricle Wall, or
Slide 36B: Right Ventricle Wall

The two types of adipose tissue are: unilocular (white) fat, which is quite common, and multilocular (brown) fat, which is rare in adults but more easily found in newborns. Unilocular (white) fat should be abundant in the epicardium on these slides. White fat can be distinguished from brown fat because each mature cell of white fat contains a single large lipid droplet in its cytoplasm while each brown fat cell contains multiple lipid droplets. White fat is therefore said to be unilocular, while brown fat is multilocular. Identify the characteristically flattened nucleus of the white adipocyte, located at the periphery of the cell in the thin rim of cytoplasm surrounding the lipid droplet. When large numbers of white adipocytes abut one another they tend to appear somewhat deformed rather than perfectly round, forming a pattern that often resembles chicken wire.

Slide 79 (HU Box): Adrenal, Human, or
Slide 47B: Primary Bronchus, Newborn, or
Slide 71B: Pancreas, Newborn

A patient search in the loose connective tissue beyond the adrenal capsule on slide 79 should reward you with some examples of brown fat. Scattered lobules of brown fat may also be present around the bronchi of the newborn in slide 47B or near the pancreas in 71B. (If you have no luck, try looking in the subcutaneous tissue of slide 30 HU (chondroid tissue). Also, about half of the 48 HU slides (fetal heart) have some brown fat around the great vessels). Observe the multiple lipid droplets within each brown fat cell, and the rounded nucleus, which is usually centrally placed in the cell rather than pushed to the side. Also notice the large number of capillaries between adipocytes (brown fat is characteristically more highly vascularized than white) and the almost glandular appearance of brown fat.
In all of these slides of brown and white fat, the lipid has been extracted by the solvents used to prepare the tissues, so the fat droplets within the adipocytes appear empty and unstained. If special fixatives had been used or the specimen had been frozen without fixation, the lipid would still be present and could be stained with appropriate stains (oil red O, osmium tetroxide, Sudan black, etc.)

B. Mucous Connective Tissue

Slide 34 (HU Box): Umbilical Cord, Human

Mucous connective tissue is a type of embryonic CT characterized by a large amount of ground substance rich in hyaluronic acid, very few fibers, and a population of undifferentiated cells. It is found primarily in the umbilical cord.

IV. CONNECTIVE TISSUE CELL TYPES

Many of these cell types are difficult to identify with your microscope unless you use the oil immersion lens. Even then it may be difficult. For good examples of each of these cell types consult the videodisk.

USE OF THE OIL IMMERSION OBJECTIVE:

The oil immersion objective is the 100X objective. It is labeled “OIL” on the side of the barrel or has a narrow black band around the barrel near its tip. To use it, proceed as follows:

1. **Without using oil**, study the section at low magnification to locate a connective tissue area for further study.
2. Continue your study with progressively stronger objectives, through 40X.
3. When you have finished observing the slide at 40X, swing that objective out of place and, observing from the side of the microscope, slowly swing the oil objective into place to be sure that it clears the coverslip.
4. Still observing from the side, raise the stage until the objective is almost touching the coverslip.
5. Then swing the oil objective out of position so that no objective is lined up with the ocular tube. Place a drop of immersion oil on the slide.
6. Carefully swing the oil immersion objective lens back into position, and slowly focus by lowering the stage with the fine focus control knob. DO NOT FOCUS BY RAISING THE STAGE SINCE IT IS EASY TO MISS FOCUS IF YOU GO TOO FAST, AND GRIND THE OBJECTIVE INTO THE COVERSIP, DESTROYING BOTH.
7. Move around the slide as much as you wish. However, if you move very far, the image may become blurry since the oil has become spread out in too thin a layer over the surface of the slide. This can be remedied by adding another drop of oil in the same manner as before.
8. When you have completed your observations with oil immersion, clean the slide and objective lens by first wiping them well with lens paper (do not use paper towels, Kleenex or any other type of paper since these may scratch the surfaces).
9. When you have finished studying the slide and have switched back to using a non-oil objective, you may find that the image appears blurry. This usually means that oil has somehow gotten onto the non-oil objective lens. Clean the lens as described in step #8.
A. Fibroblasts

Slide 34A or 34B: Mesentery

Identify the fibroblasts in the loose CT. They have an elongated, flattened, oval nucleus. Sometimes you can see the long, thin, pale-staining cytoplasmic processes of these elongated or fusiform cells, but often only the nucleus is visible. Fibroblasts synthesize virtually all the type I collagen in the body (except in the muscle layer of the wall of blood vessels, where smooth muscle cells perform this function). In connective tissues, fibroblasts produce not only the type I collagen, but also most of the other components of the extracellular matrix as well.

B. Macrophages

Slide 67 (HU Box): Liver, Stellate Reticuloendothelial Cells, India Ink, or Slide 1A: Liver, Trypan Blue Safranin

The macrophage is one of the two major phagocytic cell types in humans (the other being the neutrophil). In the preparation of these slides, the macrophages of the living animal were exposed to particulate dyes (either India ink or trypan blue) and allowed to phagocytize the dye particles prior to fixation of the tissue. The dye particles within the cytoplasmic phagosomes act as a marker for the macrophages.

The macrophages in the liver are given a special name. They are called Kupffer cells. Along with ordinary endothelial cells (which are poorly phagocytic and hence don’t take up the dye markers), the Kupffer cells line the small blood vessels of the liver called sinusoids. See if you can identify some of these dye-containing cells lining the lumen of the sinusoids.

These preparations do not preserve the details of macrophage structure very well. To study these, see the videodisk images of Kupffer cells and other macrophages. Note that most macrophages usually have an irregular nucleus (more lumpy looking rather than perfectly round or oval in outline) and a pale-staining cytoplasm. However the most reliable way to identify a macrophage by LM is to find one that contains visible phagocytized debris in its cytoplasm.

C. Mast Cells

Slide 87 (HU Box): Knee, Rat, Methylene Blue and Slide 67: Large Intestine

Search the CT of the intestinal submucosa (Slide 67) or around the knee joint (Slide 87) to find mast cells. They are usually round to oval cells that have numerous large cytoplasmic granules surrounding a round to oval nucleus that is centrally placed in the cell. The granules are usually pale-staining in H&E, but with methylene blue they may stain either dark blue or reddish-purple (metachromatic staining) depending on the vagaries of the tissue and the batch of stain. In most versions of Slide 87 the granules appear dark blue. Again, you should consult the videodisk to find high magnification images that demonstrate the detail of mast cell morphology.

When activated by appropriate stimuli, mast cells secrete histamine and certain leukotrienes that increase the permeability of small blood vessels, especially postcapillary venules. In CTs mast cells are often located near small blood vessels. See if you can confirm this relationship on Slide 67.
Basophils are leukocytes that leave the blood vessels to carry out their functions in the surrounding CTs. Both cells produce similar secretory products and have similar functions in regulating the permeability of post-capillary venules. They can be distinguished morphologically since the mast cell nucleus is round to oval and fairly easy to see within the cell, while the basophil nucleus is lobulated and often obscured by the cytoplasmic granules.

D. Lymphocytes  
Slides 9 (HU Box) or 10 (HU Box): Tonsils, or  
Slide 22: Tonsils  
The lymphoid nodules that make up the tonsils are composed mainly of lymphocytes of different sizes. Small lymphocytes have a heterochromatic nucleus that is round, slightly flattened on one side, or kidney bean-shaped. There is so little cytoplasm that it is often difficult to see it. The cytoplasm tends to be basophilic. When lymphocytes become activated during an immune reaction, they enlarge, their nucleus becomes more euchromatic, and the relative amount of cytoplasm increases. Large lymphocytes can be found in the pale staining center (called the germinal center) found in secondary lymphoid nodules (see Ross, Figs. 14.13 & 14.15a, pp. 458 & 460). Small lymphocytes are found around the edge of a secondary lymphoid nodule (the cap or mantle region), between nodules, and also infiltrating into the epithelium covering the tonsil.

E. Plasma Cells  
Slide 60 (HU Box): Duodenum. c.s., or  
Slide 61A: Fundic Stomach, Bouins, or  
Slide 62: Stomach, Pyloric, Dog, Sec. or  
Slide 67A: Colon, Bouins, or  
Slide 95A: Mammary Gland  
Search in the lamina propria beneath the epithelium of the GI tract or in the intralobular CT of the mammary gland to find plasma cells and lymphocytes. Plasma cells have an eccentric nucleus (eccentric = not in the center) with a characteristic pattern of heterochromatin clumps arranged around the periphery of the nucleus in a clockface or cartwheel arrangement. The cytoplasm of plasma cells is basophilic (a distinctive bluish-gray or lilac color) except for a pale area near the nucleus (called the cytocentrum), which contains the Golgi and centrioles. There are no large secretory granules.

F. Eosinophils  
Slide 66: Appendix  
Look in the lamina propria for eosinophils, which can be recognized by their lobed nucleus (usually bi-lobed) and numerous large secretory granules that cause the cytoplasm to be highly eosinophilic (See Wheater, Fig. 4.21, p. 81).

NOTE: Lymphocytes, eosinophils and basophils are three of the five types of white blood cells or leukocytes (neutrophils, eosinophils, basophils, lymphocytes & monocytes). All leukocytes can leave the blood vessels and enter surrounding CTs. Indeed, their major functions are carried out in the CT. Monocytes rapidly
differentiate into macrophages and so are rarely seen in the CTs. We will study their light microscope morphology when we study blood. Neutrophils and basophils are always present in CTs in small numbers, but increase greatly in number during inflammatory or allergic responses. Consult the videodisk and your atlas for light microscope images of neutrophils and basophils.

V. ELECTRON MICROSCOPY (RHODIN)
A. CONNECTIVE TISSUE FIBERS
1. Collagen fibers: Make sure you can distinguish between a collagen fiber and a collagen fibril. For most types of collagen the mature fibrils have a regular cross-banded pattern (Figs. 7-5 & 7-6). These fibrils then aggregate to form fibers that are seen in cross section and longitudinal section in Fig. 7-4. Realize that each of the small dots within a cross-sectioned fiber in this figure is a collagen fibril.
2. Elastic Fibers: Next distinguish between collagen fibrils and elastic fibers. Elastic fibers have two components: a central core of elastin and numerous microfibrils composed of fibrillin. The elastin core appears rather amorphous (literally amorphous means without shape; used to describe structures which lack distinguishing features or substructure). The microfibrils surround the elastin core, and smaller numbers may be embedded within it. Fig. 7-11 shows a single elastic fiber in cross section. Compare it with the fibrils of the collagen fiber shown at the same magnification in Fig. 7-10. Elastic fibers are usually larger in diameter than collagen fibrils, but smaller in diameter than collagen fibers. Next compare collagen and elastic fibers sectioned more longitudinally (Rhodin, Fig. 7-9). Notice again the amorphous core of the elastic fiber.
3. Reticular Fibers (Fig. 7-16): A reticular fiber is composed of reticular fibrils made of collagen type III. Reticular fibrils tend to be thinner in diameter than collagen fibrils (compare Figs. 7-10 & 7-16) and to form thinner fibers. They usually form interlacing meshworks rather than fiber bundles. In the lymphoid organs and bone marrow, they are produced by a fibroblast-like cell called the reticular connective tissue cell or reticular cell (Fig. 18-14) which is unusual in that the cytoplasmic processes of the reticular cell often remain wrapped around the reticular fibers (Fig. 18-15), to form a supporting network that actually consists of reticular fibers and reticular cells adhering to them. In most other organs, reticular fibers are produced by fibroblasts. In the tunica media of blood vessels they are made by smooth muscle cells.

B. CONNECTIVE TISSUE CELL TYPES
1. Fibroblasts are elongated fusiform (spindle-shaped or cigar-shaped) cells. When the cell is actively synthesizing and secreting proteins, the rough endoplasmic reticulum becomes quite prominent (Figs. 7-22 & 7-37), and the cisternae are often distended and irregular rather than uniformly narrow. When the cells are less active in protein synthesis, they have sparse amounts of cytoplasm with little RER or Golgi (Fig. 7-21). Some authors reserve the term fibrocyte for this less active form of the fibroblast. Note that even active fibroblasts do not contain large secretory granules since they secrete their products constitutively and do not need to concentrate them for intracellular storage.
2. Mast cells contain numerous large, electron-dense cytoplasmic granules and have a centrally placed and relatively round nucleus (Fig. 7-24). What substance is stored in the mast cell granules and then released from the activated cell to
increase the permeability of postcapillary venules? (Answer: Histamine). After
activation the cell also begins to secrete other mediators such as certain
leukotrienes that have a similar action but are not pre-synthesized and are not
stored in cytoplasmic granules. Fig. 25-16 illustrates again that mast cells are often
found near the small blood vessels (postcapillary venules and capillaries) that they
affect.

3. Macrophages develop from monocytes that have left the blood vessels. Macrophages (Fig. 7-26) have the characteristics you would expect from highly
phagocytic cells: a well-developed Golgi complex (where the lysosomes are
formed), numerous primary lysosomes, secondary lysosomes, residual bodies,
phagosomes, and an active plasmalemma, i.e., one with many projections of the
surface membrane that are engaged in the formation of phagosomes (these are labeled "microvilli" in this micrograph).

4. Mature adipocytes in white fat are unilocular, with the nucleus compressed
peripherally in the thin rim of cytoplasm surrounding the large lipid droplet (Fig. 7-
39). Observe that when lipid is preserved it appears dark by electron microscopy
(Fig. 7-39), and when it has been extracted during fixation, the lipid droplets are
light-staining (Figs. 7-40 & 7-41). Notice (Figs. 7-40 to 7-42) that during their
development the adipocytes of white fat pass through a multilocular stage (i.e.,
each cell has numerous lipid droplets which later coalesce into one droplet). Brown
fat, in contrast, is always multilocular.

5. Lymphocytes are small cells when not activated in an immune response (Fig. 17-
23). A small lymphocyte has a heterochromatic nucleus and very little cytoplasm.
The cytoplasm is basophilic due to the presence of free polysomes, but other
organelles are few in number. After a lymphocyte has been activated in an immune
response it enlarges (Fig. 17-24) the nucleus becomes more euchromatic, the
relative amount of cytoplasm increases, and the various cytoplasmic organelles
become more abundant.

6. Plasma cells (Fig. 7-29) develop from B lymphocytes during an immune response. They have clumps of heterochromatin margined along the inner surface of the
nuclear membrane, abundant rough endoplasmic reticulum (which accounts for the
basophilia of the cytoplasm by light microscopy) and a well-developed Golgi. The
cartwheel or clockface pattern of heterochromatin is not always as apparent in
electron micrographs as in light micrographs. What protein is being synthesized on
the RER of a plasma cell? (Answer: Immunoglobulins, otherwise known as
antibodies).

7. Eosinophils (Fig. 5-12) are another type of leukocyte. They have a lobed nucleus
(usually bi-lobed), and numerous large cytoplasmic granules. We have seen that
by light microscopy they can be distinguished from mast cells by the fact that the
mast cell nucleus tends to be round or oval, and mast cell granules are highly
basophilic while those of the eosinophil are eosinophilic (hence the name of the
cell). By electron microscopy the two cell types can be distinguished by their
nuclear morphology and the fact that eosinophil granules tend to contain a
crystalloid (Fig. 5-13) while those of mast cells do not. The crystalloid contents
include a protein called major basic protein, which has anti-parasitic activity.
NOTE: The lymphocyte and eosinophil are two of the five types of white blood cells or leukocytes (neutrophils, eosinophils, basophils, lymphocytes & monocytes). All leukocytes can leave the blood vessels and enter surrounding CTs. Indeed, their major functions are carried out in the CT. The detailed ultrastructure of the other leukocytes will be considered when we study blood.

C. CONNECTIVE TISSUE TYPES

1. Mesenchyme (Figs. 7-1 & 7-2): Mesenchyme is an embryonic form of CT. Note that the extracellular matrix consists of abundant ground substance and relatively few fibers. The fibers that are present tend to be very thin reticular fibers. The mesenchymal cells have multiple long, thin cytoplasmic processes and they make up a homogeneous population (i.e., virtually all the cells have the same undifferentiated appearance). The cells somewhat resemble fibroblasts, but if they were mature fibroblasts you would expect to see more fibers in the matrix and at least a few differentiated cells of other types (lymphocytes, mast cells, macrophages, etc.).

2. Loose cellular connective tissue (Fig. 7-31): Characterized by large numbers of cells, many different cell types and relatively few thin fibers.

3. Loose connective tissue vs. dense irregular connective tissue (Fig. 7-32): Compare the number of cells per unit area and the thickness of the collagen fibers in the upper portion (loose CT) vs. the lower portion (dense CT) of this micrograph.
**LABORATORY 3 CHECKLIST**  
**CONNECTIVE TISSUE PROPER & ADIPOSE TISSUE**

### LIGHT MICROSCOPY

<table>
<thead>
<tr>
<th>Structure</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>fibroblast</td>
<td>Kupffer cell</td>
</tr>
<tr>
<td>collagen fibers</td>
<td>mast cell</td>
</tr>
<tr>
<td>lamina propria</td>
<td>small lymphocyte</td>
</tr>
<tr>
<td>tendon</td>
<td>large lymphocyte (in germinal center)</td>
</tr>
<tr>
<td>papillary layer of the dermis</td>
<td>plasma cell</td>
</tr>
<tr>
<td>reticular layer of the dermis</td>
<td>eosinophil</td>
</tr>
<tr>
<td>elastic fibers</td>
<td>loose connective tissue</td>
</tr>
<tr>
<td>elastic lamellae</td>
<td>loose cellular connective tissue</td>
</tr>
<tr>
<td>reticular fibers</td>
<td>dense irregular connective tissue</td>
</tr>
<tr>
<td>white fat</td>
<td>dense regular connective tissue</td>
</tr>
<tr>
<td>brown fat</td>
<td>reticular connective tissue</td>
</tr>
<tr>
<td>macrophage</td>
<td>mucous connective tissue</td>
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</tbody>
</table>

### ELECTRON MICROGRAPHS

<table>
<thead>
<tr>
<th>Structure</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>collagen fibril</td>
<td>macrophage</td>
</tr>
<tr>
<td>collagen fiber</td>
<td>white adipocyte</td>
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<tr>
<td>elastic fiber</td>
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<tr>
<td>fibroblast</td>
<td>eosinophil</td>
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<tr>
<td>mast cell</td>
<td></td>
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</tbody>
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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 3: CONNECTIVE TISSUE PROPER & ADIPOSE TISSUE

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. What is the major identifying characteristic that allows you to classify a
tissue as a connective tissue?

2. What is the major identifying characteristic that allows you to classify a
tissue as an epithelium?

3. What are the major functions of connective tissue?

4. Which type of connective tissue (loose, dense irregular or dense regular)
would be best suited for resisting strong forces that are consistently applied
in the same direction?

5. What cell type is responsible for the synthesis of collagen in connective
tissues? In the muscle layer of the wall of blood vessels?

6. Under what circumstances does the number of “wandering” or “immigrant”
cells in a CT increase dramatically?

7. What color do most trichromes stain collagen? What color do most stain
smooth muscle?

8. What tissue component is stained by aldehyde fuchsin?

9. What stain is most commonly used to demonstrate reticular fibers?

10. Reticular fibers are PAS positive. What color would they be after PAS
staining? What information do you gain from the fact that they are PAS
positive whereas other types of collagen fibers are not?

11. What does the term “reticular” tell you about the arrangement of reticular
fibers in a tissue?

12. Is there a membrane surrounding each individual lipid droplet in the
cytoplasm of brown or white fat cells?

13. Can lipid droplets stain black in an electron micrograph? Can they stain gray
or white?

14. Why don’t plasma cells have large secretory granules in their cytoplasm?

15. Protein secreting cells such as plasma cells often have a pale cytoplasmic
area called the centrosome (cytocentrum). What organelle is
characteristically found in the centrosome and how is it related to protein
synthesis and secretion?
LABORATORY 4
INTEGUMENT

OBJECTIVES:

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

1. the layers of the epidermis and dermis
2. the cell types of the epidermis: keratinocyte, melanocyte, Langerhans cell and Merkel cell
3. the process of keratinization, and the morphological evidence of this process: tonofilaments and keratohyalin granules
4. how thick skin differs morphologically from thin skin
5. the histology of integumentary appendages: eccrine and apocrine sweat glands, sebaceous glands, hair follicles, and nails
6. morphological differences at the LM and EM level between the integument and minimally keratinized stratified squamous epithelia

LABORATORY:

The skin is one of the largest organs of the body, accounting for 15-20% of its total mass. In this lab you will have the opportunity to apply much of what you learned in previous labs about epithelium and connective tissue and to see how tissues are combined to form an organ system. You will examine the surface epithelium (epidermis) and connective tissue layer (dermis) of the skin and the eccrine sweat glands, apocrine sweat glands and sebaceous glands. You will learn about hair follicles and nails, which are specialized derivatives of the epidermis.

A. Thick Skin
   Slide 54 (HU Box): Skin, Thick
   Slide 40A: Skin, Cornified Layer
   Slide 41A: Glabrous Skin, or
   Slide 41B: Plantar Skin H&E
   and
   Slide 41: Plantar Skin, Masson Aldehyde-Fuchsin
   Be sure to study both the H&E and trichrome-stained preparations.

   Thick skin is limited to the palm of the hands and the soles of the feet. In classifying skin as either thick or thin, it is the thickness of the epidermis that is important. The thickness of the dermis is irrelevant. Thick skin is hairless (glabrous), while thin skin in most locations is hairy (vellus).
Skin (whether it is thick or thin) consists of an epidermis and an underlying dermis. The epidermis is a maximally keratinized stratified squamous epithelium in which the major cell type is the keratinocyte.

Identify the five layers of the epidermis in thick skin: stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum. The stratum basale is a single layer of cuboidal or columnar cells that rests on the basement membrane. It contains stem cells that will divide and differentiate into the keratinocytes of the other layers. For this reason it is sometimes called the stratum germinativum. (Some mitotic cells can also be found in the lower parts of the stratum spinosum, and some texts will include this region in the definition of the stratum germinativum.)

In slide 41A identify the melanin and note that it is present mainly in the stratum basale. Melanin is made by melanocytes, which are scattered, between the cells of the stratum basale (see Ross, Fig. 15.7, p. 498 & Wheater, Fig. 9.6a, p. 166). They have elongated cytoplasmic processes (visible only by EM) that extend up into the stratum spinosum. Melanin is contained within membrane-bounded organelles (melanosomes) derived from the Golgi of the melanocytes. Melanosomes are rapidly transferred from melanocytes to keratinocytes, so that most melanocytes are actually rather pale-staining, and most of the melanin is within keratinocytes. Melanocytes are neural crest derivatives; they do not differentiate from the stem cells of the stratum basale.

The stratum spinosum is a relatively thick layer containing polygonal cells in its lower portion and more flattened cells in its upper portion. The cells are separated from one another by an intercellular space of relatively uniform width. This space is thought to be a shrinkage artifact caused by fixation. The keratinocytes are joined together by many small spinous processes that are barely visible at 40x. Each spine represents a site where a desmosome connects two tiny cytoplasmic processes, one from each of the neighboring cells. These numerous desmosomes give the epithelium great mechanical strength and make this a wear and tear epithelium.

The stratum granulosum is only a few cells thick. The cells contain highly basophilic cytoplasmic granules called keratohyalin granules. These are involved in the process of keratinization. The stratum granulosum is the most superficial layer to contain live, nucleated keratinocytes.

The stratum lucidum is usually observed only in thick skin. It is no more than a few cells layers thick. The cells are usually anucleate and are refractile or slightly eosinophilic.

In the stratum corneum the cells are anucleate and are flatter than those in the deeper layers. In the upper portions of the stratum corneum, the desmosomes connecting keratinocytes break down, and thin sheets or squames of cells peel off and are shed (desquamated). Note that maximally keratinized stratified squamous epithelia are distinguished from minimally keratinized stratified squamous by the appearance of the surface cells. Maximally keratinized cells are very flat, anucleate, and dead whereas minimally keratinized cells are plumper, contain nuclei and are still living.

The epidermis is supported by the dermis, a layer of fibroelastic connective tissue. Immediately under the epidermis is the papillary layer of the dermis, which is made up of loose connective tissue. Deep to this is the reticular layer, which is composed of dense irregular connective tissue. Notice how the junction between the epidermis and dermis is thrown into folds; the downward folds of the epidermis are called rete pegs (rete ridges, epidermal pegs, epidermal ridges). They interdigitate with the upward projections of the dermis called dermal ridges or dermal papillae (forming the papillary layer of the dermis). The dermal papillae often contain capillary loops, and function as a means of carrying the blood supply to the more superficial layers of the avascular epithelium.
Deep to the dermis is the loose connective tissue of the hypodermis, which consists largely of adipose tissue. Be able to identify the eccrine sweat glands. The secretory portion of the gland is a coiled tube located deep in the dermis or in the upper portion of the hypodermis. Find the myoepithelial cells associated with the secretory portion. The unbranched duct of the sweat gland travels through the dermis, and then takes a spiral course as it penetrates the epidermis.

B. Thin Skin & Hair

Slide 40: Skin Caucasian, & Skin, Bouins,
Slide 42: Scalp, and
Slide 44: Eyelid

Examine slide 40 or 42 first. As in thick skin, thin skin also has a stratified squamous epithelium that is maximally keratinized. However, in thin skin the epidermis has only four easily visible layers since the stratum lucidum is not evident by LM. All the remaining layers tend to be thinner than in thick skin, but the stratum corneum is the most dramatically reduced. Identify the stratum basale, stratum spinosum, stratum granulosum and stratum corneum on these slides. Often the stratum granulosum is only one cell thick. Notice that in some slides the keratinocytes of the stratum basale contain visible amounts of melanin produced by melanocytes.

Thin skin usually contains hair follicles. Slide 42 of the scalp should contain many examples. Look for a longitudinal section through a follicle and identify the hair bulb, dermal papilla, external root sheath, internal root sheath, hair shaft, and connective tissue root sheath. The hair bulb is the wide lower end of the follicle. It is located in the lower part of the dermis or in the hypodermis. The deep surface of the hair bulb is invaginated by the dermal papilla, which is highly vascularized connective tissue and is required for maintenance of the follicle. Many of the epithelial cells in the hair bulb are stem cells called matrix cells that give rise to the hair shaft itself and to the internal root sheath. See if you can find any mitotic figures in this region. The internal root sheath degenerates as the cells move toward the surface of the epidermis. It has disappeared by the time you reach the level where the sebaceous glands empty into the hair follicle. This leaves a space between the hair shaft and the external root sheath into which the secretions of the sebaceous glands (sebum) can be released. Scattered between the matrix cells of the hair bulb are the melanocytes that are responsible for hair color.

The external root sheath is a downgrowth of the epidermis. It is separated from the connective tissue root sheath by a thick basement membrane called the glassy membrane, which may be visible at high mag in very good preparations. The glassy membrane is continuous with the basement membrane of the epidermis. The connective tissue root sheath is a condensation of the connective tissue of the dermis. Find some cross sections through different levels of hair follicles and try to identify as many of these same features as possible. In a cross section of a hair shaft see if you can distinguish the centrally located medulla of the shaft from the more heavily pigmented cortex. Only thick hair shafts have a medulla. In addition, the outermost layer of the shaft (the cuticle) is often quite thin and difficult to visualize by light microscopy.

Just for fun, try to decide if the scalp in your slide 42 came from an individual with straight or curly hair. The shafts of straight hairs are more likely to be round in cross section rather than oval, and the follicles tend to be oriented almost perpendicular to the surface of the skin rather than more obliquely oriented.

Identify an arrector pili muscle. These are bundles of smooth muscle cells that originate in the papillary layer of the dermis and insert into the connective tissue root.
sheath of the hair follicle. Their contraction causes the hair to become more erect, and by pulling on the skin also causes the slight dimpling we call goosebumps.

Associated with the hair follicles are sebaceous glands. These acinar glands have a very short duct that usually opens into a hair follicle just superficial to the attachment of the arrector pili muscle. Review the structure of these glands and see Ross Fig. 15.13, p. 505 for an illustration of the overall structure of a sebaceous gland and its relationship to a hair follicle. On slide 44 of the eyelid find the modified sebaceous gland called a Meibomian gland. This consists of many sebaceous acini opening into a very long duct. About 20-25 Meibomian glands open directly onto the margin of an eyelid, just posterior to the eyelashes.

On slide 44, examine the inner surface of the eyelid. This stratified squamous epithelium lines a moist surface, and is therefore minimally keratinized.

Slide 99 (HU Box): Skin, Human, Pigmented & Non-Pigmented, Elastic Stain

Compare the amounts of pigmentation in these two sections of thin skin. In the heavily pigmented skin it may be possible to see that the melanin tends to lie between the nucleus of the cell and the surface of the skin, as if protecting the DNA of the nucleus from harm.

Be aware that the amount of pigmentation varies between regions of the body even in the same individual. This is due to variation in the number of melanocytes/mm$^3$ in the different locations. However, if you compare the pigmentation of the same region (e.g., the back of the hand) in individuals of different races, the number of melanocytes per mm$^3$ would be strikingly similar in both individuals. The racial differences in pigmentation at a comparable location are determined by how many melanosomes each melanocyte produces, how much melanin they contain, and how rapidly they are degraded. It is not due to differences in the number of melanocytes. Notice that in the heavily pigmented skin there is evidence that the melanin persists longer since the melanin granules are still visible in layers located closer to the surface of the skin.

C. Apocrine Sweat Glands
Slide 53 (HU Box): Lip, Skin (Thin)

Slide 53 (HU) is a non-human primate lip. It contains apocrine sweat glands which are not present in human lip but which are essentially identical to the apocrine sweat glands found in other characteristic locations in human skin (e.g., the axilla, areola and nipple of the mammary gland, perianal skin, etc.). Study their morphology here in the lip. Apocrine sweat glands are coiled tubular glands. The secretory portion can be distinguished from an eccrine sweat gland because the apocrine gland characteristically has a much wider lumen surrounded by a simple cuboidal to columnar layer of secretory cells. The apical cytoplasm often bulges into the lumen producing a scalloped edge at the lumen of the gland. Myoepithelial cells are present around the secretory portion. The duct of apocrine glands is similar to that of eccrine sweat glands. Although it is usually not obvious in sections, apocrine glands open into hair follicles superficial to the point where the sebaceous gland empties.

Also note the large hair follicles with large thin-walled blood sinusoids surrounding the root. They are specialized sensory receptors known as sinus hairs. Humans do not have sinus hairs. You may also note the skeletal muscle fibers inserting into the dermis, possibly from the orbicularis oris muscle, a muscle of facial expression.
Slide 17 (HU Box): Recto-Anal Junction, l.s.
Identify apocrine sweat glands, sebaceous glands, and hair follicles in this non-human specimen.

D. Nails
Slide 10: Finger, or
Slide 30 (HU Box): Chondroid tissue, human
Both of these slides show fetal fingers. The nail is not present on all versions of these slides. If you can locate one, identify the nail plate, nail bed, nail root, matrix, eponychium (cuticle), and hyponychium. The nail plate is the nail itself. The nail bed is the epithelium upon which the nail plate rests. The stem cells that give rise to the nail plate are found at its proximal end in an area called the nail root or matrix. The eponychium is the cuticle and the hyponychium is the ridge of skin under the free end of the nail that joins the nail to the skin. Pulling the hyponychium away from the nail plate is extremely painful.

ELECTRON MICROSCOPY (RHODIN)
Keratinocytes:
The major cell type of the epidermis is the keratinocyte. The ultrastructure of the keratinocyte is different in each layer of the epidermis, mainly due to the ongoing process of keratinization. Identify the layers in LMs (Figs. 25-1 & 25-2) and in EMs of thin skin (Figs. 25-4, 25-9 & 25-17). Now observe how the structure of keratinocytes differs in each layer:

1. In the stratum basale keratinocytes are cuboidal to columnar. They rest on the basement membrane and are anchored to it by hemidesmosomes (Figs. 25-4, 15-7 & 25-8). They are connected to one another and to the cells of the stratum spinosum by desmosomes (Fig. 25-5 & 25-7). Their cytoplasm contains intermediate filaments (Fig. 25-6 & 25-8) called tonofilaments that begin to organize into bundles. These bundles become thicker and more numerous in the stratum spinosum and granulosum. Free ribosomes are abundant in the cytoplasm.

2. In the stratum spinosum (Figs. 25-4 to 25-6) the cells are more polygonal or slightly flattened, and have many spines. The spines represent sites where neighboring cells are connected by desmosomes. Note the large tonofilament bundles and the numerous free ribosomes.

3. Keratohyalin granules are first produced in the upper part of the stratum spinosum, but are most abundant in the stratum granulosum. As part of the keratinization process, they disperse in the stratum lucidum or stratum corneum, and coat the tonofilaments (Figs. 25-4 & 25-9 to 25-11).

4. Production of membrane-coating granules (MCGs) or lamellar bodies also begins in the upper stratum spinosum, but like keratohyalin granules, MCGs are more numerous in the stratum granulosum. They are visible only by EM, but are poorly demonstrated in these micrographs. Fig. 25-12 is a high magnification view of one MCG that is in the process of releasing its contents into the extracellular space by exocytosis. This occurs at the interface between the granulosum and the lucidum or corneum. The MCGs contain a mixture of lipids that coats the outer surface of
the cell membrane and helps to establish a barrier that “waterproofs” the epidermis to prevent excess fluid loss from the body.

5. The **stratum corneum** (Figs. 25-4, 25-9 & 25-11 to 25-13) contains very flat cells whose nucleus and cytoplasmic organelles are gone. The bundles of tonofilaments have dispersed and the cells are now packed with keratin composed of individual tonofilaments embedded in a matrix contributed by the keratohyalin granules.

**NOTE:** There is no example of lucidum since these micrographs are from thin skin.

Skin contains other cell types in addition to keratinocytes. Study the following:

1. **Melanocytes** (Figs. 25-17 & 25-18): Melanocytes sit on the basement membrane and have numerous elongated cytoplasmic processes that extend between cells of the stratum basale and stratum spinosum. They produce melanin in membrane-bounded structures called **melanosomes**, and transfer these structures to surrounding keratinocytes. The enzyme tyrosinase is essential to this process.

2. **Langerhans cells** (Fig. 25-17) are antigen-presenting cells involved in initiating immune responses. They are derived from stem cells in the bone marrow. They are found mainly in the stratum spinosum where they can be identified by their pale cytoplasm that contains neither keratin nor melanosomes. Note that they are not melanocytes without melanosomes as Rhodin states. They are unrelated to melanocytes. Langerhans cells can leave the epidermis and migrate to regional lymph nodes, carrying antigens on their surface that will initiate an immune response in the node.

3. **Merkel cells** (no illustrations in Rhodin; see Fig. 15.10 in Ross) are located mainly in the stratum basale. They are associated with terminal expansions of some of the free nerve endings and are thought to be involved in the sense of touch. They contain small dark-staining vacuoles that tend to be clustered near the nerve ending. Note that of these three cell types (melanocytes, Langerhans cells & Merkel cells) only Merkel cells are attached to keratinocytes via desmosomes.

Identify the **basal lamina** in Figs. 25-7 & 25-8. What is the difference between a basal lamina and a basement membrane?

Study the ultrastructure of the following integumentary appendages:

1. **Eccrine sweat glands and their ducts** (Figs. 25-45 to 25-48, 25-51 & 25-53): In the secretory portion of the gland note the dark cells, clear cells and myoepithelial cells. Rhodin refers to the dark cells as “mucoid”. This is somewhat misleading, since they do not have the morphology of mucous cells. Their nuclei do not become flattened at the base of the cell, and their secretory product is not easily extracted, so their cytoplasm does not appear pale-staining and empty by light microscopy. In fact, they have the morphology of fairly typical serous cells. The epithelium that lines the ducts is a two-layered stratified cuboidal epithelium. The ducts lack myoepithelial cells.

2. **Apocrine sweat glands** (Figs. 25-56 to 25-58): Note the wider lumen than in the eccrine glands, the scalloped edge of the lumen in actively secreting glands, and the myoepithelial cells. The dark granules that Rhodin identifies as either secondary lysosomes or secretory granules (#6 in Fig. 25-58) are now considered to be secretory granules.

3. **Sebaceous glands** (Figs. 25-40 to 25-42): In Fig. 25-42 identify the stem cells at the periphery of the gland, and observe how the cytoplasm gradually fills with lipid.
droplets as the cells mature and move toward the duct. The contents of the lipid droplets are partially extracted in these micrographs. There are no myoepithelial cells associated with the secretory or duct portions of sebaceous glands.

4. Hairs and hair follicles (Figs. 25-23 to 25-29). In these light micrographs taken from different levels through the hair shaft and hair follicle, identify the dermal papilla, hair bulb (called hair papilla in Rhodin), medulla and cortex of the hair shaft, inner root sheath, outer root sheath, and the connective tissue sheath.

   Observe that the inner root sheath keratinizes and degenerates in the upper portion of the follicle. Also, the glassy membrane is not easily visible in these micrographs, but is located between the outer root sheath and the connective tissue sheath.

   Compare these views with the EM of the hair at midshaft as seen in Fig. 25-30. The layers labeled #4 & #5 in this micrograph are both part of the outer root sheath.

### LABORATORY 4 CHECKLIST

#### INTEGUMENT

**LIGHT MICROSCOPY**

<table>
<thead>
<tr>
<th>thick vs. thin skin</th>
<th>hair follicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>epidermis vs. dermis</td>
<td>hair bulb</td>
</tr>
<tr>
<td>keratinocyte</td>
<td>external root sheath</td>
</tr>
<tr>
<td>stratum basale</td>
<td>internal root sheath</td>
</tr>
<tr>
<td>stratum spinosum</td>
<td>hair shaft</td>
</tr>
<tr>
<td>stratum granulosum</td>
<td>glassy membrane</td>
</tr>
<tr>
<td>stratum lucidum</td>
<td>arrector pili muscle</td>
</tr>
<tr>
<td>stratum corneum</td>
<td>sebaceous gland</td>
</tr>
<tr>
<td>melanin</td>
<td>apocrine sweat gland</td>
</tr>
<tr>
<td>melanocyte</td>
<td>nail plate</td>
</tr>
<tr>
<td>papillary vs. reticular layer of dermis</td>
<td>nail bed</td>
</tr>
<tr>
<td>hypodermis</td>
<td>eponychium</td>
</tr>
<tr>
<td>secretory part vs. duct of eccrine sweat gland</td>
<td>hyponychium</td>
</tr>
<tr>
<td>myoepithelial cell</td>
<td>nail root or matrix</td>
</tr>
</tbody>
</table>

**ELECTRON MICROGRAPHS**

<table>
<thead>
<tr>
<th>keratinocyte</th>
<th>melanosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>hemidesmosome</td>
<td>Langerhans cell</td>
</tr>
<tr>
<td>stratum basale</td>
<td>eccrine sweat gland</td>
</tr>
<tr>
<td>stratum spinosum</td>
<td>apocrine sweat gland</td>
</tr>
<tr>
<td>desmosome</td>
<td>sebaceous gland</td>
</tr>
<tr>
<td>melanocyte</td>
<td></td>
</tr>
</tbody>
</table>

**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 4: INTEGUMENT
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. What criterion determines whether skin is classified histologically as thick or thin skin? Is it the thickness of the epidermis, the dermis, or the epidermis + dermis together?

2. Where is thick skin found?

3. What is glabrous vs. vellus skin? How do these terms relate to thick and thin skin?

4. Which layer of the epidermis is present in thick skin but is missing or difficult to see by light microscopy in thin skin?

5. In a stratified epithelium such as the epidermis, where are the stem cells located?

6. Does a simple epithelium have stem cells too?

7. Which of the four cell types in the epidermis is/are not derived from the epidermal stem cells?

8. Where are Merkel cells located? What is their function?

9. What is the major chemical component of keratohyalin granules?

10. Why are keratohyalin granules visible in the stratum granulosum but not in the stratum lucidum or corneum?

11. In slides that are stained with aldehyde fuchsin-Masson (e.g. slide 41), the stratum corneum is black. What chemical component causes this staining?

12. How and by what cell type are membrane-coating granules produced? How are their contents released from the cell?

13. What is the function of membrane-coating granules?

14. What enzyme can be used as a marker for melanocytes and why?

15. Which type of skin gland is characteristically not associated with hair follicles?

16. In which layer of the epidermis are Langerhans cells usually located? What unique organelle do they contain?

17. What do you think causes hair to turn gray or white in old age?

18. Is male pattern baldness caused by the death of the hair follicles?

19. The type of touch receptor found in the hypodermis or deep in the dermis is a _____________ (Pacinian corpuscle, Meissner’s corpuscle, Merkel cell).

20. As a second degree burn heals (i.e., one that destroys the full thickness of the epidermis but spares the dermis), where do the epithelial cells that eventually re-cover the surface of the dermis come from?

21. Can you tell the difference between eccrine and apocrine sweat glands based on a cross section through their ducts?
LABORATORY 5
PLACENTA & MAMMARY GLAND

OBJECTIVES:

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

1. recognize and describe the structure and functions(s) of:
   - chorionic plate (fetal side of the placenta)
   - decidua basalis (maternal side of placenta)
   - chorionic villi (primary, secondary & tertiary)
   - stem villi
   - anchoring villi
   - syncytiotrophoblast
   - cytotrophoblast
   - Hofbauer cells
   - decidual cells
   - maternal blood space
   - amnion

2. distinguish between an early and a late placenta
3. recognize the umbilical cord, umbilical arteries & umbilical vein
4. describe the general histology of the mammary gland
5. distinguish between the inactive mammary gland of the non-pregnant female, the proliferating gland of pregnancy, and lactating gland
6. describe the modes of secretion of the alveolar cells during lactation

LABORATORY:

Study the following slides:

I. PLACENTA (See diagram at the end of this section)

Slide 98: Placenta

The placenta has a fetal component called the chorion frondosum and a maternal component called the decidua basalis. This slide shows only chorion frondosum, which consists of the chorionic plate and the chorionic villi that extend from it. The chorionic plate can be distinguished from the decidua basalis by several criteria including:

1. The pale appearance of the chorionic plate, which reflects the highly hydrated nature of the ground substance in fetal tissue.

2. Larger blood vessels (which are main branches of the umbilical arteries and vein) may be visible in the chorionic plate; most of the maternal blood vessels in the decidua basalis tend to be much smaller.

3. Early in development the fetal red blood cells are nucleated. Later in development they will be anucleate. Red blood cells in the maternal blood vessels of the decidua basalis are not nucleated at any time. Therefore if you see nucleated erythrocytes in a blood vessel, you know you are looking at the fetal portion of an early placenta.
The chorionic villi are initially composed of a core of cytotrophoblast cells covered by the syncytiotrophoblast. Such villi are called primary villi. Soon mesenchyme extends into the villi to form their cores, and the villi are then called secondary villi. Finally fetal blood vessels grow into the mesenchyme, and the villi are then called tertiary villi. Primary and secondary villi are present only very early in development, so the great majority of the villi on these slides are tertiary. Within the mesenchymal core of some villi it is possible to identify Hofbauer cells. These are usually the only spherical cells in the mesenchyme. They have a spherical nucleus, which is usually centrally located, and a pale-staining lacy cytoplasm. They are macrophages, and may also play a role in angiogenesis within the villi.

The chorionic villi project from the chorionic plate into the intervillous space (maternal blood space). Both the chorionic plate and the villi are lined by cytotrophoblast and syncytiotrophoblast, with the syncytiotrophoblast being in direct contact with the maternal blood in the intervillous space. Maternal blood enters the intervillous space via the spiral arteries of the uterine endometrium. Exchange between fetal and maternal blood occurs across the syncytiotrophoblast, cytotrophoblast, mesenchyme of the villus, and endothelium of fetal capillary.

Slide 98A: Placenta, 2 Months
Slide 99: Placenta, 4 1/2 Months
Slide 100 (HU Box): Placenta, Human

(NOTE: Slide 99 shows a somewhat earlier stage of development than Slides 98A or 100HU. Also, some versions of Slide 99 have two serial sections of the same placenta side by side. Do not be confused and think that one is the fetal side and the other the maternal side. Both are fetal side).

All these slides show later placentas than Slide 98. The features that allow you to conclude this include:

1. The villi are more highly branched.
2. Fewer fetal red blood cells are nucleated.
3. The layer of cytotrophoblast cells has become discontinuous in places. This is due to the fact that cytotrophoblast cells are fusing with the syncytiotrophoblast faster than they are replicating to produce more cytotrophoblast. Eventually virtually all the cytotrophoblast cells will fuse with the syncytiotrophoblast.

Slide 100: Placenta, and
Slide 100A: Placenta, Late

Both of these slides are of late placenta, which differs from the midterm placenta in the following ways:

1. The villi are even more extensively branched and more closely packed within the maternal blood spaces.
2. The cytotrophoblast is almost completely gone.
3. “All” fetal red cells are non-nucleated. The nuclei you may see within fetal vessels belong to fetal leukocytes.
4. Most fetal blood vessels have moved closer to the periphery of the villi so that they are located just beneath the syncytiotrophoblast. This has the advantage of decreasing the diffusion distance that nutrients & wastes must travel between the fetal & maternal circulation.

5. In many places the nuclei of the syncytiotrophoblast cluster together to form syncytial knots (trophoblastic knots). This leaves long stretches of syncytiotrophoblast where only a thin layer of syncytiotrophoblast cytoplasm separates the fetal blood vessels from the maternal blood space. This is thus another mechanism for minimizing the thickness of the maternal-fetal blood barrier. Syncytial knots frequently pinch off from the villi and enter the maternal circulation. Some of these become emboli in the maternal blood and lodge in the maternal lung. They seem to cause relatively few clinical problems.

6. There is an increase in the amount of a very eosinophilic material called fibrinoid in the extracellular space of the chorionic plate, the villi and especially the maternal side of the placenta.

Slide 100HU and most versions of Slide 100 show both sides (maternal and fetal) of the placenta. The maternal component of the placenta is the decidua basalis, which is the portion of the uterine wall to which the anchoring villi of the fetal component attach. In this slide distinguish the maternal side of the placenta (decidua basalis) from the fetal side (chorionic plate), using the following criteria:

1. The paler appearance of the fetal side due to its highly hydrated ground substance.
2. The large blood vessels in the chorionic plate.
3. The presence of decidual cells on the maternal but not the fetal side of the placenta.
4. The amnion lies immediately adjacent to the fetal side in the late placenta. It is a simple low cuboidal epithelium, and is visible on some versions of Slide 100. It is located on the opposite side of the chorionic plate from the maternal blood space.
5. The larger amount of fibrinoid associated with the maternal side.

Decidual cells are evident in the decidua basalis on Slide 100. They are large, pale, rounded cells with a relatively large amount of cytoplasm. They are derived from stromal cells of the uterus (i.e., maternal connective tissue). By LM they cannot be distinguished from peripheral cytotrophoblast cells, which are also present in the decidua basalis. Peripheral cytotrophoblast cells are derived from the cytotrophoblast of anchoring villi. They eventually form a continuous layer (the outer cytotrophoblast shell). This makes up the interface with the maternal connective tissue (in the endometrial layer of the uterus).

Any large villus that is directly continuous with the chorionic plate is a stem villus. Any villus that is directly continuous with the decidua basalis is an anchoring villus. If you could follow an anchoring villus from the decidua basalis all the way back to the chorionic plate, you would find that it was also a stem villus.

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II. UMBILICAL CORD

Slide 34 (HU Box): Umbilical Cord, Human, c.s.

This is a cross section through the umbilical cord of a full term fetus. Note the mucoid connective tissue (Wharton’s jelly) making up the core of the cord. The amnion forms the outer epithelial covering of the umbilical cord. Distinguish between the two umbilical arteries and one umbilical vein. The arteries have more orderly walls in which you can more easily see the difference between the smooth muscle layer (the tunica media) and the outer layer (the tunica adventitia), which contains connective tissue. The umbilical arteries carry deoxygenated blood from the fetus to the placenta. Each develops from one of the two dorsal aortas of the fetus. The umbilical vein carries oxygen-rich blood from the placenta to the fetus. It is called a vein because it returns blood to the fetal heart.

III. MAMMARY GLAND

The mammary glands of a reproductive age female may be classified as:
1. inactive or resting
2. proliferating during pregnancy
3. lactating
Inactive or resting:
The lobules in an inactive breast consist of a relatively small number of ducts with relatively few alveolar buds. The alveoli cannot be readily distinguished from cross sectioned ducts. The ducts and alveoli within a lobule are surrounded by loose intralobular connective tissue, while the lobules are separated from one another by considerable amounts of dense interlobular connective tissue. The inactive glands of a mature female resemble the glands of a prepubertal female except that in the mature individual there is more adipose tissue and the ducts have more branches.

Pregnancy:
During pregnancy there is rapid growth and branching of the ducts, and development of alveoli made up of secretory cells. In comparison with the inactive breast, each lobule now appears larger and more crowded with cross sections of ducts and alveoli. The amount of connective tissue (especially fat) between lobules decreases, thus forming thinner interlobular septa. It is still difficult to distinguish ducts from secretory alveoli unless the ducts are cut in longitudinal section, revealing their tubular rather than spherical shape. Plasma cells become more numerous in the intralobular connective tissue, since they are producing the antibodies that are secreted into the colostrum and then the milk.

Lactating:
In lactating mammary glands there is an extreme proliferation of secretory alveoli. This causes the lobules of the gland to increase so much in size that they replace most of the interlobular connective tissue, making it difficult to see the septa between lobules. The alveoli become greatly distended by eosinophilic secretory material (colostrum and then milk; colostrum actually begins to accumulate in late pregnancy so that there is a ready supply of food for the infant immediately after birth). It is not unusual for the appearance of neighboring alveoli to vary considerably; some may have a cuboidal epithelium and a relatively small lumen while adjacent alveoli have a more squamous epithelium and a much wider lumen.

Please study as many of the following slides of the mammary gland as you can, and determine whether they represent the inactive, pregnant or lactating condition. Do not rely on the labels on the slides, since “active” mammary gland may refer to either the pregnant or lactating condition, and some of the slides simply appear to be mislabeled. Also, not all versions of the same slide number show mammary glands at the same stage, making it impossible for us to give you a list of which slide number shows what.

Slide 20 (HU Box): Mammary Gland, Inactive, Human
Slide 95A: Only the version labeled “Mammary Gland, Human H9.351”
Slide 96: Breast, Bouins, H&E
Slide 96A: Mammary Gland, Human
Slide 97 (Two Versions): Mammary Gland or Mammary Gland during Pregnancy
Slide 95: Active Breast
Slide 95A: Only those labeled “Mammary Gland Form, H&E Homo 28 Yrs”
Slide 21 (HU Box): Mammary Gland, Active
Slide 96B: Mammary Gland, Human, Active
IV. ELECTRON MICROSCOOPY (RHODIN)

PLACENTA

Study the structure of a tertiary villus (Figs. 34-57 to 34-59). Identify syncytiotrophoblast, cytotrophoblast cells, fetal blood vessels, and maternal blood space. Realize that the syncytiotrophoblast is a true syncytium (i.e., a multinuclear cell formed by the fusion of uninucleate cells). In Fig. 34-58 observe that the cytotrophoblast layer has already become discontinuous in places. Part of a Hofbauer cell (identifiable because of the lysosomes and phagosomes in its cytoplasm) is visible in this figure. Review the layers of the placental barrier (maternal-fetal blood barrier) shown in Fig. 34-59, and understand that later in pregnancy this barrier would be even thinner due to the absence of cytotrophoblast. In Figs. 34-57 & 34-58, distinguish between fetal and maternal red blood cells.

MAMMARY GLAND

Compare the light micrographs of resting (Fig. 34-63), proliferating (Fig. 34-64) and lactating (Fig. 34-65) mammary glands at low mag.

Study the alveoli of the lactating gland (Figs. 34-67 & 34-68). Notice that the milk in the lumen has two different morphologically recognizable components: large lipid droplets and small dark-staining protein particles (mainly casein). The protein particles are contained within membrane-bounded vacuoles in the cytoplasm (Fig. 34-70) and are released by merocrine secretion. In contrast, the lipid droplets in the cytoplasm have no limiting membrane, and are released by apocrine secretion (Fig. 4-24). As a part of this secretory process the lipid droplet acquires a membrane by budding through the plasma membrane. Also notice in Fig. 34-68 that the secretory alveoli are surrounded by myoepithelial cells. Myoepithelial cells can be identified by EM because of their content of actin filaments, which are involved in contractility. These cells are often difficult to identify by LM.
# LABORATORY 5 CHECKLIST
## PLACENTA & MAMMARY GLAND

### LIGHT MICROSCOPY

<table>
<thead>
<tr>
<th>Structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>chorionic plate</td>
<td>decidua basalis</td>
</tr>
<tr>
<td>amnion</td>
<td>decidual cells</td>
</tr>
<tr>
<td>tertiary villus</td>
<td>anchoring villus</td>
</tr>
<tr>
<td>stem villus</td>
<td>umbilical cord</td>
</tr>
<tr>
<td>cytotrophoblast</td>
<td>umbilical artery</td>
</tr>
<tr>
<td>syncytiotrophoblast</td>
<td>umbilical vein</td>
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<tr>
<td>fetal capillaries in placenta</td>
<td>Wharton’s jelly</td>
</tr>
<tr>
<td>Hofbauer cells</td>
<td>inactive mammary gland</td>
</tr>
<tr>
<td>syncytial knots</td>
<td>proliferating mammary gland of pregnancy</td>
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<tr>
<td>maternal blood space</td>
<td>lactating mammary gland</td>
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<tr>
<td>early vs. late placenta</td>
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</table>

### ELECTRON MICROGRAPHS

<table>
<thead>
<tr>
<th>Structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>tertiary villus</td>
<td>layers of maternal/fetal blood barrier</td>
</tr>
<tr>
<td>syncytiotrophoblast</td>
<td>apocrine secretion of milk lipids</td>
</tr>
<tr>
<td>cytotrophoblast</td>
<td>merocrine secretion of milk proteins</td>
</tr>
<tr>
<td>maternal blood space</td>
<td>myoepithelial cells of mammary gland</td>
</tr>
<tr>
<td>fetal capillaries in placenta</td>
<td></td>
</tr>
</tbody>
</table>

**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 5: PLACENTA & MAMMARY GLAND

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. When syncytial knots pinch off of placental villi and enter the maternal circulation, why do they preferentially end up in the maternal lung rather than in some other organ?

2. Are all anchoring villi part of a stem villus?

3. Through what vessel(s) does fetal blood enter the placenta? Trace its pathway within the placenta. Through what vessel(s) does it leave, and where does it go?

4. How does maternal blood enter and leave the placenta? What is its pathway within the placenta?

5. Within the placenta, maternal blood is always in contact with and confined by what cell layer?

6. As the placenta matures during pregnancy, what structural changes occur that result in increased efficiency of oxygen exchange between fetal and maternal blood?

7. Name some structural features that allow you to distinguish the maternal side of the placenta from the fetal.

8. What is a placental cotyledon?

9. What is the difference between a primary, secondary and tertiary placental villus?

10. Does a late placenta have primary and secondary villi as well as tertiary?

11. During pregnancy, why do plasma cells become more numerous in the intralobular connective tissue of the mammary gland?

12. Why can’t all the components of milk (lipid and proteins) be released by merocrine secretion?

13. Myoepithelial cells contract to help move secretory product into or along the duct system of a gland. In the lactating mammary gland, are the myoepithelial cells stimulated to secrete by direct innervation or by a hormonal signal?
LABORATORY 6

MUSCLE

OBJECTIVES:

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

1. distinguish by light microscopy between the three types of muscle, in both longitudinal and cross-section:
   - skeletal muscle
   - cardiac muscle
   - smooth muscle

2. distinguish by electron microscopy between the three types of muscle

3. understand the organization of actin and myosin myofilaments in the sarcomeres of cardiac and skeletal muscle, how this accounts for specific bands, and how the bands change during muscle contraction

4. distinguish between myofilament, myofibril, muscle fiber and muscle fascicle

5. identify endomysium, perimysium and epimysium

LABORATORY:

Please study the following slides in your set:

I. SKELETAL MUSCLE
   Slide 43 (HU Box): Skeletal Muscle, Entire, c.s., and
   Slide 14A: Skeletal Muscle, c.s. & l.s.

Skeletal muscle cells are called muscle fibers (myofibers). They are long, unbranched cylindrical cells, oriented parallel to the long axis of the muscle. They are multinucleate, with numerous flattened nuclei located at the periphery of the cell just beneath the plasmalemma (sarcolemma). The cytoplasm of an individual cell contains many myofibrils, each of which is a bundle composed of many myofilaments. The myofilaments are made of actin or myosin, plus associated proteins.

Regular cross-striations are characteristic feature of both skeletal and cardiac muscle (which are therefore collectively referred to as striated muscle). The striations should be visible in good H&E preparations of longitudinally cut muscle fibers. Identify the alternating light bands (I-bands) and dark bands (A-bands). These result from the arrangement of actin and myosin myofilaments. Probably not visible in these slides are the dark lines called Z-lines that bisect each I-band. The basic contractile unit is the sarcomere. A sarcomere extends from one Z-line to the next.

Connective tissue is found in characteristic locations within muscle tissue, and is given different names based on its location. The endomysium is a small amount of connective tissue including reticular and collagenous fibers located between individual muscle cells. Fibroblasts and capillaries can be seen within the endomysium. The perimysium is the connective tissue surrounding each muscle fascicle (bundle of muscle cells), and the epimysium is the connective tissue sheath surrounding the surface of the entire muscle.
Slide 14B: Skeletal Muscle, Fe-He
This section was stained with iron-hematoxylin, which makes the A-bands and I-bands easily visible, and sometimes reveals the Z-lines as well.

II. CARDIAC MUSCLE
Study two or three of the following:
Slide 36: Left Ventricle Wall
Slide 36B: Right Ventricle Wall
Slide 39: Heart Muscle LS
Slide 48 (HU Box): Entire Heart, I.S.
Slide 44 (HU Box): Cardiac Muscle
Cardiac muscle is found in the middle layer of the heart, which is called the myocardium. In cardiac muscle individual cells are arranged end to end to form a branching fiber. Cardiac muscle cells, like skeletal muscle fibers have sarcomeres with regular cross-striations. However, as seen in the table below, there are several major differences that should help you distinguish cardiac and skeletal muscle.

<table>
<thead>
<tr>
<th>FEATURE</th>
<th>CARDIAC MUSCLE</th>
<th>SKELETAL MUSCLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cell diameter</td>
<td>Intermediate between smooth &amp; skeletal</td>
<td>Largest of all muscle types</td>
</tr>
<tr>
<td>2. Nuclear position</td>
<td>Centrally located</td>
<td>Peripherally located</td>
</tr>
<tr>
<td>3. Number of nuclei</td>
<td>1 or 2</td>
<td>Multinucleate (&gt;2)</td>
</tr>
<tr>
<td>4. Intercalated discs</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>5. Fibers</td>
<td>Branched</td>
<td>Unbranched</td>
</tr>
</tbody>
</table>

One feature diagnostic of longitudinally sectioned cardiac muscle cells is the intercalated disc. Intercalated discs (most easily visible in Slide 39). You may find it helpful to close down the condenser diaphragm &/or decrease the light intensity to best observe these structures. They represent membrane junctions that join successive cardiac muscle cells to one another end-to-end. These junctions are located at the level of Z lines. Between two successive intercalated discs lies an entire cardiac muscle cell including many sarcomeres. Intercalated discs appear as cross-bands that are darker and more prominent than Z lines, A bands or I bands. They sometimes appear to travel straight across the muscle cell, but in other cases they may have a step-like appearance. We will see the explanation for this step-like phenomenon when we examine these cells by electron microscopy. In regions where the muscle cells are longitudinally sectioned, see if you can identify any binucleate cells.

Locate some cardiac myocytes in cross section in order to observe the centrally located nuclei, and the much smaller average diameter of cardiac muscle cells vs. skeletal muscle. Note also the numerous capillaries between myofibers. Cardiac muscle is normally more vascular than skeletal or smooth muscle.
III. SMOOTH MUSCLE

Individual smooth muscle cells are fusiform (cigar-shaped or spindle-shaped), being thickest near the centrally located nucleus and tapering at the ends. Smooth muscle cells lack the visible striations seen in skeletal and cardiac muscle, and have the smallest average cell diameter of the three muscle types.

Slide 63 (HU Box): Colon, or
Slide 67A: Colon, Bouins

Find an area of the slide where the smooth muscle cells have been cut in cross section and confirm the much smaller average diameter of smooth muscle cells compared to skeletal or cardiac muscle. Notice the central location of the nuclei. Also observe that in cross-sectioned smooth muscle there is a large range of cell diameters since some cells will have been cut through the thick central portion of the cell near the nucleus and others through the narrow ends of the cells. This difference in diameter between adjacent cells cut in cross section is more pronounced for smooth muscle than for cardiac or skeletal muscle (where the cells tend to be more cylindrical and lack tapering ends). This feature helps identify smooth muscle in cross section.

In the wall of the colon, as in many other tubular viscera, the smooth muscle cells are organized into layers that appear to be oriented perpendicular to one another. The colon contains an inner circular layer (nearest the lumen) and an outer longitudinal layer. This arrangement will permit you to examine smooth muscle cells cut longitudinally and in cross section within the same tissue section.

Slide 32A: Renal Artery, or
Slide 4 (HU Box): Artery, Vein & Nerve, Elastic Stain

Confirm that the wall of an artery or vein contains one layer of smooth muscle (the tunica media), and that the muscle cells in that layer are circularly arranged relative to the lumen of the vessel. Essentially all the nuclei you see in the tunica media belong to smooth muscle cells. The tunica media is unusual in that this is one location where smooth muscle cells rather than fibroblasts produce most of the extracellular matrix, including elastic and collagen fibers.

Slide 29 (HU Box): Uterus, Secretory Phase, or
Slide 92: Uterus - Early Proliferative

Find the extensive smooth muscle layer (myometrium) of this organ. Notice that in comparison to the smooth muscle of the gut and blood vessels, which is organized in well-ordered layers, the smooth muscle of the uterus is organized into small interlacing bundles that are oriented in many different directions. This is typical of spherical, rather than tubular organs. This interlacing arrangement provides a muscle layer with no major connective tissue planes along which separations might occur. In the uterus this allows the organ to better withstand the strong contractions of parturition without damage.

Slide 17 (HU Box): Recto-Anal Junction, I.s.

Surrounding the recto-anal junction is a thickened layer of smooth muscle (the internal or involuntary anal sphincter), and an outer layer of skeletal muscle (the external or voluntary anal sphincter). You can locate the area of interest by identifying the region where the simple columnar epithelium of the rectum changes into the minimally
stratified squamous epithelium of the lower anal canal. Then look at the muscle layers in this general region and make certain you can distinguish the smooth muscle from skeletal muscle and connective tissue. You can locate the internal anal sphincter because it is continuous with the inner circular layer of smooth muscle in the muscularis externa of the gut. It is innervated by the autonomic nervous system. The external anal sphincter lies further away from the lumen of the gut. Since it is composed of skeletal muscle, is innervated by somatic motor neurons (the alpha motor neurons of the spinal cord).

IV. ELECTRON MICROSCOPY

A. The Sarcomere

Fig. 11-5 shows a longitudinal section of skeletal muscle. Identify the A band, the I band & the Z line. The light-staining zone that bisects the A band is the H zone (from the German “helle” or light). Realize that a sarcomere runs from Z line to Z line and hence includes the A band and half of two different I bands. Observe the peripheral nuclei of the skeletal muscle cell (#2) and realize how careful you have to be to distinguish them from the nuclei of endothelial cells (#3) that lie just exterior to the muscle cell. This micrograph shows parts of four different muscle cells. Appreciate that the cytoplasm is filled with myofibrils created by arranging sarcomeres end to end. The myofibrils are closely but not perfectly aligned with one another. At higher magnification you would be able to see that there is a small amount of cytoplasm, including a moderate number of mitochondria, between myofibrils (see Ross, Figs. 11.5 & 11.7, pp. 315 & 318).

Fig. 11-10 shows a high mag view of two myofibrils and reveals more detail of the sarcomere. Now you can appreciate that the I band contains the thin myofilaments composed mostly of actin. They are anchored in the Z line. Thin filaments extend from the Z line into the A band where they interdigitate with the thick filaments (composed mostly of myosin) that define the A band. You can also see that the H zone represents the region of the A band where thin and thick filaments do not overlap (only thick filaments are present). At the center of the H zone is the M line, where thick filaments are crosslinked by proteins that help keep them in register.

B. Skeletal Muscle

Be able to identify the sarcoplasmic reticulum or (SR). This is visible in Rhodin Figs. 11-10 & 11-15, but is better appreciated in Wheater, Figs. 6.8 & 6.9, pp. 106 & 107. The SR is the specialized smooth endoplasmic reticulum of striated muscle cells. It forms a network of irregular anastomosing tubules surrounding each myofibril. After contraction calcium is pumped from the cytosol into the SR tubules, inhibiting actin and myosin interaction and thus causing contraction to stop.

Find the T tubules (again best seen in Wheater, Figs. 6.8 - 6.9, pp. 106-107). Note that T tubules are invaginations of the plasma membrane (sarcolemma) whereas the SR membranes are not continuous with the sarcolemma at all. T tubules carry the depolarization of the sarcolemma deep into the interior of the cell to facilitate contraction. In skeletal muscle they are located near the junction of the A & I bands. In Wheater, Fig. 6.8 or Ross, Fig. 11.8 note that the T tubules and the SR both surround each myofibril.
within the muscle cell. In skeletal muscle each T tubule is usually flanked on each side by a distended portion of the SR called a terminal cisterna. A T tubule plus the two terminal cisternae that flank it form a unit known as a triad, which plays an important role in excitation-contraction coupling. The illustration in Wheater (Fig. 6.8, p.106) gives a particularly good 3-dimensional view of triads. In a longitudinal section of the muscle cell (Wheater, Fig. 6.9), the T tubules and terminal cisternae of the triads often look like vesicles, but when the muscle cell is cut in cross section (Wheater, Fig. 6.10) the ring-like arrangement of the triads around each myofibril sometimes becomes more evident.

Motor neurons innervate skeletal muscle cells at myoneural junctions (motor end plates) (Figs. 11-18 & 14-20). The motor neurons associated with skeletal muscle are myelinated. As they approach the muscle, the myelin sheath of the nerve fiber ends (Fig. 14-20), although a layer of Schwann cell cytoplasm persists as the teloglia that covers the dilated axonal ending. This axonal ending sits in a trough (the primary synaptic cleft) in the surface of the muscle cell. The axonal ending can be identified by its numerous clear synaptic vesicles containing neurotransmitter (Fig. 14-21), and by its abundant mitochondria, whereas actin and myosin myofibrils may sometimes be visible in the muscle cell cytoplasm (Figs. 11-18 & 14-20). The sarcolemma of the muscle cell forms numerous junctional folds that create smaller subneural clefts (secondary synaptic clefts) that open into the primary cleft. The junctional folds are diagnostic for synapses between nerve and skeletal muscle.

C. Cardiac muscle

Fig. 11-25 illustrates the width of a cardiac muscle cell (indicated by the bar) and the central location of the nucleus. Compare this with the greater cell width and peripheral nuclei of skeletal muscle (Fig. 11-3). Notice that at either pole of the nucleus in the cardiac muscle cells there is an area of cytoplasm where myofibrils are absent (Figs. 11-25 & 11-26). This region contains the Golgi apparatus. The rest of the cytoplasm is packed with myofibrils made up of sarcomeres stacked end to end. The banding pattern of the sarcomere is the same in both skeletal and cardiac muscle. Between the myofibrils numerous mitochondria are packed in rows. One feature that helps distinguish cardiac from skeletal muscle is that cardiac muscle contains many more mitochondria than skeletal.

A second distinguishing feature of cardiac muscle is the presence of intercalated discs (Figs. 11-25 & 11-27). These occur where the sarcolemma of one muscle cell abuts end-to-end with the next cell. A cardiac muscle cell therefore extends longitudinally from one intercalated disc to the next. Note that the myofibrils within one cell are not all of equal length. As a result, the ends of the cell have an irregular serrated or step-like appearance. A single intercalated disc follows these irregularities and therefore has transverse and longitudinal portions. The transverse portions are parallel to the Z lines and the longitudinal portions are parallel to the long axis of the myofibrils. The longitudinal parts of an intercalated disk include gap junctions that are responsible for the electrical coupling of cardiac muscle cells. The transverse portions of a disk contain desmosomes and fasciae adherentes that provide the mechanical strength to hold neighboring cardiac muscle cells together. A fascia adherens (plural=fasciae adherentes) also serves as the site of attachment for actin filaments, and hence can be thought of as a substitute for a Z line and as a relative of the zonula adherens found in epithelial cells. These various junctions are visible in Rhodin, Figs 11-29 & 11-30, but Wheater, Fig. 6-26, p. 118 is a better illustration.
Identify the T tubules of cardiac muscle and the terminal cisternae of the sarcoplasmic reticulum (Figs. 11-32 & 11-33). In Fig. 11-33 two terminal cisternae flank the T tubule, forming a triad, but triads are actually less common in cardiac muscle than in skeletal. More often cardiac muscle has only diads (a T tubule and one terminal cisterna). This reflects the fact that the sarcoplasmic reticulum is less extensive in cardiac muscle so there are fewer terminal cisternae. Notice that the diads & triads of human cardiac muscle are located at the Z line rather than near the A-I junction as in skeletal muscle.

D. Smooth muscle

Figs. 11-39, 11-40, & 11-42 show two features that are characteristic of smooth muscle cells: dark-staining structures in the cytoplasm called dense bodies (fusiform densities), and numerous micropinocytotic vesicles (also called caveolae) that are invaginations of the plasma membrane. The dense bodies are unique to smooth muscle. They contain alpha-actinin, and thin filaments are thought to attach to them. Therefore dense bodies are somewhat analogous to Z-lines. In most sections they appear to be small separate structures scattered throughout the cytoplasm or attached to the plasma membrane, but serial sections show that they are actually long branching strands that form a network throughout the cytoplasm and attach to the plasma membrane. They anchor actin filaments as well as other components of the cytoskeleton (desmin and vimentin intermediate filaments). The pinocytotic vesicles or caveolae apparently contain calcium channels that allow calcium to enter the cell when it is stimulated to contract. They also interact with sparse cisternae of SER that lie just beneath the membrane to release stored calcium from the SER into the cytosol. In this respect the caveolae function much like T-tubules.

Note also that smooth muscle cells, like those of cardiac muscle have a centrally placed nucleus (Fig. 11-39), but the smooth muscle cells have a smaller average diameter. Cytoplasmic processes from one smooth muscle cell contact neighboring cells and establish intercellular communication via gap junctions (Fig. 11-39), which make coordinated contraction of smooth muscle cells possible. Note that smooth muscle does contain thick filaments as well as thin actin filaments (Fig. 11-45 & 11-47), but these are quite fragile and special fixation methods are often necessary to preserve them. The thick and thin filaments are not arranged into sarcomeres and thus smooth muscle does not appear striated by light microscopy.

Finally study the innervation of smooth muscle (Fig. 11-50, 14-25 & 14-26). Smooth muscle is innervated by postganglionic autonomic neurons. In comparison to skeletal muscle, the myoneural junctions of smooth muscle are much less elaborate. Observe that:

1. The sarcolemma of smooth muscle cell has no junctional folds.
2. The distance between the nerve terminal and the muscle is often much greater in smooth muscle than in skeletal muscle. Neurotransmitters must diffuse greater distances to reach the smooth muscle cell.
3. A single nerve terminal can innervate several smooth muscle cells (Fig. 11-50). This is in contrast to the one-to-one relationship between nerve terminal and muscle cell in skeletal muscle (Figs. 11-17 & 18). Neurotransmitters released from one smooth muscle synapse can therefore cause contraction of multiple muscle cells.
The dense-cored vesicles in Fig. 11-50 contain noradrenergic transmitters (noradrenaline), which is typical of postganglionic sympathetic neurons. Many smooth muscle cells also receive parasympathetic innervation. The postganglionic parasympathetic nerve endings would have clear synaptic vesicles that contain acetylcholine. Acetylcholine is also the usual neurotransmitter at preganglionic sympathetic and parasympathetic endings, and at somatic motor nerve endings on skeletal muscle.

If you could study serial sections along an autonomic axon innervating smooth muscle, you would see that the synaptic regions of the nerve are not restricted to the end of the axon as they are in somatic motor nerves. Instead there are multiple synapses along the length of an axon, each one forming a bulge or varicosity on the axon. These varicosities are called endings “en passant” meaning “in passage”.

**LABORATORY 6 CHECKLIST**

**MUSCLE**

LIGHT MICROSCOPY

<table>
<thead>
<tr>
<th>skeletal vs. cardiac vs. smooth muscle in cross section</th>
<th>skeletal vs. cardiac vs. smooth muscle in longitudinal section</th>
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</thead>
<tbody>
<tr>
<td>sarcomere</td>
<td>endomysium</td>
</tr>
<tr>
<td>A band</td>
<td>intercalated disc</td>
</tr>
<tr>
<td>I band</td>
<td></td>
</tr>
</tbody>
</table>

ELECTRON MICROGRAPHS

<table>
<thead>
<tr>
<th>skeletal vs. cardiac vs. smooth muscle in longitudinal section</th>
<th>diad of cardiac muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>thin filaments</td>
<td>myoneural junction of skeletal muscle</td>
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<tr>
<td>thick filaments</td>
<td>junctional folds</td>
</tr>
<tr>
<td>I band</td>
<td>primary synaptic clefts</td>
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<tr>
<td>A band</td>
<td>synaptic vesicles</td>
</tr>
<tr>
<td>Z line</td>
<td>intercalated disc</td>
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<tr>
<td>H band</td>
<td>dense bodies of smooth muscle</td>
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<tr>
<td>sarcoplasmic reticulum</td>
<td>caveolae of smooth muscle</td>
</tr>
<tr>
<td>terminal cisternae</td>
<td>myoneural junction of smooth muscle</td>
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<tr>
<td>T tubule</td>
<td></td>
</tr>
<tr>
<td>triad of skeletal muscle</td>
<td></td>
</tr>
</tbody>
</table>

**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 6: MUSCLE

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. What is the distinction between a structural syncytium and a functional syncytium? Is skeletal muscle a structural or a functional syncytium? Cardiac muscle? Smooth muscle?

2. What specific name is given to the layer of connective tissue that immediately surrounds each muscle cell? Each fascicle (bundle) of muscle cells? An entire muscle?

3. What is the relationship between a myofibril, a sarcomere, and a myofilament?

4. During contraction of skeletal or cardiac muscle, do the I bands shorten or remain constant in length? The A bands?

5. Suppose you were looking at a cross section of a sarcomere by EM. If you saw only thick filaments, and these filaments appeared to be cross-linked to one another, you could conclude that this cross section had passed through what part of the sarcomere (Z line, I band, zone of thick-thin overlap in the A band, H zone, or M-line)?

6. In skeletal and cardiac muscle, is the sarcoplasmic reticulum located between myofibrils or between neighboring muscle cells?

7. Into which type of junction in an intercalated disk do the actin filaments of cardiac muscle insert? This junction is analogous to what component of the junctional complexes that are found between epithelial cells?

8. Name two characteristic features visible by EM in smooth muscle that distinguish it from skeletal or cardiac muscle.

9. If you were looking at a cross section of the colon, would the individual smooth muscle cells of the inner circularly arranged muscle layer be cut longitudinally or in cross section?
LABORATORY 7

NERVE

OBJECTIVES:

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

1. the basic organization of the neuron (nerve cell), including cell body (soma), dendrites, axon, and synapses
2. the structure of a synapse, including pre- and postsynaptic elements
3. the morphological differences between dorsal root (spinal) ganglia vs. autonomic and enteric ganglia, and the neurons they contain
4. the basic organization of the spinal cord and the motor neurons it contains
5. the two types of glia in the peripheral nervous system: satellite cells and Schwann cells
6. the structure of a peripheral nerve, including the morphology of the myelin sheath, and the relationship of Schwann cells to axons and to myelin
7. the endoneurium, perineurium, and epineurium
8. the structure and function of selected motor and sensory nerve endings including the:
   - neuromuscular junction of skeletal muscle
   - muscle spindle
   - Pacinian corpuscle
   - Meissner’s corpuscle

LABORATORY:

The nervous system is divided anatomically into central nervous system or CNS made up of the brain and spinal cord, and the peripheral nervous system or PNS, which includes all nerve tissue outside the CNS (peripheral nerves, spinal (dorsal root) ganglia, autonomic ganglia, and the enteric nervous system). The neuron is the fundamental cell type of the nervous system, and is found in both the CNS and the PNS. Since the CNS will be studied in the Neuroscience course, we will limit our study mainly to the PNS.

Depending on their function and location, neurons vary considerably in size, shape, and in the branching pattern of their cell processes, especially their dendrites. Characteristics shared by most neurons include a large, round euchromatic nucleus, a prominent nucleolus, and a basophilic cytoplasm. The cytoplasm includes the perikaryon (Latin = around the nucleus) and a variable number of processes. The perikaryon and the nucleus together form the cell body or soma. The neuronal processes usually include a single axon arising from a region of the perikaryon known as the axon hillock, and one or more dendrites. Axons and dendrites are often difficult to follow for any distance in H&E preparations, but with special stains (for example, certain silver stains or the Golgi technique), the branching details of these processes can be better appreciated. Another feature of neurons that can be demonstrated with special stains is
Nissl substance (Nissl bodies). A Nissl body is a stack of several short cisternae of RER, with free polysomes scattered between cisternae. Nissl bodies are located in the perikaryon (except for the region of the axon hillock) and extend into dendrites but not into the axon.

Neuronal cell bodies outside the CNS are located in clusters, each of which is called a ganglion. Spinal ganglia (also called dorsal root ganglia) contain the cell bodies of sensory neurons. Autonomic ganglia contain the cell bodies of postganglionic sympathetic or parasympathetic neurons. They are by definition visceral motor neurons and innervate smooth muscle, cardiac muscle or glands. Enteric ganglia contain the cell bodies of enteric neurons, which are the intrinsic neurons of the GI tract. They include motor and sensory neurons.

A peripheral nerve includes one or more bundles or fascicles of nerve fibers. A nerve fiber is a neuronal process (either a peripheral process of a sensory neuron or an axon of a motor neuron) plus the Schwann cells associated with it. A nerve fiber may be myelinated or unmyelinated. Schwann cells form the myelin sheaths in the PNS. Regardless of whether they are myelinated or not, all the nerve fibers in a peripheral nerve include Schwann cells. A peripheral nerve also includes connective tissue elements called the epineurium, perineurium and endoneurium (see below).

In addition to neurons, the nervous system contains various types of accessory cells called neuroglia or glia. The CNS and PNS contain morphologically different types of glia. In the PNS the glia include satellite cells, which are in direct contact with the neuronal somas, and the Schwann cells associated with the neuronal processes. In the CNS the glia include oligodendrocytes (which produce the myelin sheaths of the CNS), astrocytes, microglia and ependymal cells. The CNS glia will be studied in the Neuroscience course.

Please study the following slides in your set:

I. SPINAL MOTOR NEURONS
Slide 93 (HU Box): Nissl Bodies, Neurocytes, Nissl Method

This slide shows a cross section of spinal cord (see Wheater, Fig. 20.10, p. 392 for orientation). Identify the white matter, which contains the processes of neurons (unstained by the Nissl method), and glial cells (small cells whose cytoplasm is difficult to see). You do not need to distinguish between types of CNS glia in this course. Find the more centrally located gray matter, which is shaped somewhat like a butterfly and contains the central canal of the spinal cord. The gray matter is organized into a dorsal (posterior) horn and a ventral (anterior) horn on either side of the cord. The ventral horn can be identified by the presence of very large motor neurons. These are the alpha motor neurons, which innervate skeletal muscle. Observe their euchromatic nucleus and a prominent nucleolus. Find the highly basophilic Nissl bodies in their cytoplasm (see Wheater, Fig. 7.4b&c, p.126). Search your section to see if it has a good example of a neuron where the axon hillock is visible as a pale-staining region of the perikaryon, i.e., an area that lacks Nissl bodies. Look for the axon arising from the axon hillock. Alpha motor neurons are multipolar neurons that have one axon and many dendrites. Identify a dendrite using the fact that the initial portion of a dendrite contains Nissl bodies. The axon of these cells leaves the spinal cord in the ventral root and usually branches to end on many skeletal muscle cells. Note that the gray matter also includes glial cells as well as smaller neurons, some of which may be interneurons that form connections between neurons within the spinal cord.
II. GANGLIA OF THE PERIPHERAL NERVOUS SYSTEM

A. Spinal Ganglia (Dorsal Root Ganglia)

The spinal (dorsal root) ganglia contain the somas (cell bodies) of sensory neurons. They are derived from neural crest. These ganglion cells have the euchromatic nucleus, prominent nucleolus and relatively large amount of basophilic cytoplasm that is typical of neurons. Notice that these cells are so large that they may sometimes appear to be anucleate. Distinguish the neurons from the two types of smaller non-neuronal cells found in ganglia: satellite cells, which directly surround the neuronal perikaryon, and Schwann cells, which surround the nerve processes. Generally the nucleus of both these cell types is smaller and more heterochromatic than that of the neuron, and it is difficult to locate the boundaries (plasma membranes) of the cell.

Although not immediately apparent in sectioned material, the neurons of the spinal ganglia are pseudounipolar, meaning that only one process extends out from the cell body. It then divides into a peripheral process and a central process. The peripheral process ends (in skin, joints, skeletal muscle, tendons, viscera, etc.) either as a free nerve ending or in association with a peripheral sensory receptor (e.g. Pacinian corpuscle, Meissner’s corpuscle, muscle spindle). Collectively these different types of endings sense parameters such as touch, temperature, pain, distension, vibration, etc. The central process travels in the dorsal roots to enter the dorsal (posterior) horn of the spinal cord and deliver the sensory input to the central nervous system.

Slide 41 (HU Box): Fetus, Rat, Sagittal Section

Look for developing spinal ganglia. They are located in the intervertebral foramina of the developing vertebrae of the spinal column. To find the ganglia you must have a section that is slightly off the midsagittal plane of the embryo, since a section through the midsagittal plane would show only the spinal cord, not the more laterally placed ganglia. Notice that a well-organized layer of satellite cells has not yet developed around the ganglion cells at this stage.

B. Sympathetic Ganglia

Search for sympathetic ganglia in the loose connective tissue surrounding the large mesenteric artery. Sympathetic and parasympathetic ganglia are part of the autonomic nervous system. They contain postganglionic autonomic neurons that are motor (rather than sensory) to targets including smooth muscle, the cardiac conduction system, and glands (they are secretomotor to glands, i.e. they stimulate secretion). Within an autonomic ganglion, preganglionic neurons whose cell bodies are in the spinal cord synapse on the postganglionic neurons.

You should be able to distinguish the sensory neurons in a spinal ganglion from the neurons in an autonomic ganglion. Autonomic ganglion cells (sympathetic or parasympathetic) tend to be smaller, often have an eccentric nucleus rather than a centrally placed one, and are multipolar (one axon and many dendrites), rather than pseudounipolar. In sections it is not always evident that the cells are multipolar. Instead, what you may notice is that the layer of satellite cells seems much less complete around autonomic neurons than around pseudounipolar sensory neurons. This is because the ring of satellite cells is interrupted in many more places by the numerous processes arising from the multipolar autonomic neuron.
We can guess that the ganglia on this slide are sympathetic rather than parasympathetic because of their location. The slide label tells us that these ganglia are located near one of the mesenteric arteries. You will learn in Gross Anatomy that such ganglia are normally sympathetic.

C. Enteric Ganglia

Slide 3 (HU Box): Myenteric (Auerbach’s) Plexus, Intestine, Mallory Trichrome, or Slide 67: Large Intestine

The enteric nervous system is the intrinsic nervous system of the GI tract. By saying that this system is intrinsic to the gut we mean that all enteric neurons are contained entirely within the gut wall, including their cell bodies, their axons and all their dendrites. Enteric neurons are located in many small ganglia that are part of two plexuses located in different layers of the gut wall. These are the myenteric plexus (Auerbach’s plexus) and the submucosal plexus (Meissner’s plexus). The myenteric plexus is located between the inner and outer smooth muscle layers of the muscularis externa, whereas the submucosal plexus is located in the connective tissue layer called the submucosa. Morphologically, enteric neurons resemble those of sympathetic or parasympathetic ganglia, although they may be somewhat smaller. Unmyelinated nerves interconnect the ganglia within each plexus, and also connect the two plexuses with one another.

Enteric neurons control peristalsis and other movements of the gut. They can function without any input from the CNS, as evidenced by the fact that peristalsis can occur even in isolated gut segments where all CNS input has been cut. However the activity of enteric neurons is normally modulated by input from sympathetic and parasympathetic neurons. For this reason, the enteric nervous system is often considered to be a third division of the autonomic nervous system. Sympathetic activity normally causes a decrease in peristaltic contractions while parasympathetic innervation causes an increase.

The neurons of enteric ganglia include sensory neurons that receive input from sensory endings in the wall of the gut, and motor neurons that innervate the smooth muscle cells in the gut and are also secretomotor to intestinal glands. These sensory and motor neurons are not morphologically distinguishable from one another by light microscopy.

The enteric plexuses are ensheathed by connective tissue. In the Mallory trichrome (Slide 3 HU), note the blue-green collagen fibers around the ganglia in the myenteric plexus. The cytoplasm of any neuron anywhere in the PNS or CNS, especially in older individuals, may accumulate a brownish pigment called lipofuscin. This represents indigestible material located in residual bodies. In some versions of these slides the enteric neurons contain lipofuscin. See Wheater, Fig. 1.25a, p. 30 for an example of lipofuscin within the neurons of a sympathetic ganglion.

III. Peripheral Nerve

Slide 95 (HU Box): Medullated Nerve, c.s. & l.s., PAS-Orange G
(Note: “Medullated” is an outdated term meaning “myelinated”.)

A peripheral nerve consists of many sensory and motor nerve fibers that carry information between the CNS and the rest of the body. The term “nerve fiber” is used here to refer to a neuronal process (an axon of a motor neuron or a process of a sensory
neuron) plus all the Schwann cells that are associated with it. In myelinated fibers, each Schwann cell forms a segment of the elaborate wrapping (the myelin sheath) that surrounds each axon individually. Unmyelinated fibers in the PNS are still associated with Schwann cells, but there are multiple axons associated with each Schwann cell rather than just one, and the Schwann cell membrane does not wrap repeatedly around these axons to form myelin. Instead, each axon occupies an invagination in the membrane of the Schwann cell.

A peripheral nerve may contain myelinated fibers, unmyelinated fibers or a mixture of both. Most versions of this slide show a mixed nerve with myelinated and unmyelinated fibers. Do peripheral nerves contain the nuclei of neurons? (Answer: No. The nuclei of neurons are either in the CNS or in ganglia. The nuclei that you see in peripheral nerve belong mostly to Schwann cells.)

Myelin is largely lipid, thus standard fixation and dehydration methods leave a pale-staining “foamy” residue where the lipid of the myelin sheath would be in life. The neuronal process is usually visible as a pink strand at the center of the nerve fiber. Be careful to distinguish this from the cytoplasm of the Schwann cell, most of which is located at the outer surface of the myelin sheath. This outer collar of Schwann cell cytoplasm can be identified because it is also the location of the Schwann cell nuclei, whereas you will not find any nuclei in the neuronal process itself. Notice that the myelin sheaths are not all the same thickness. Some fibers are heavily myelinated while others are more lightly myelinated, even within the same nerve fascicle. Identify nodes of Ranvier in the longitudinal view. They represent the regions between two Schwann cells that are myelinating adjacent segments along the length of one neuronal process.

Most peripheral nerves contain both motor and sensory processes. They cannot be distinguished from one another morphologically. Both motor and sensory processes can be unmyelinated, lightly myelinated or heavily myelinated.

In the cross-section, locate the endoneurium, perineurium and epineurium. The endoneurium consists of the delicate collagenous and reticular tissue surrounding each nerve fiber and is difficult to see by LM unless you have a trichrome-stained section. The perineurium in contrast is very obvious. It is formed by one or more layers of tightly packed, flattened cells that surround each fascicle of nerve fibers. Large nerves may contain many of these fascicles, while the smallest nerves contain only one. The cells of the perineurium are distinctive by electron microscopy since they have characteristics of smooth muscle cells and of epithelia. They are connected by tight junctions that help form a “blood-nerve barrier” that regulates the environment within the fascicle and promotes the transmission of action potentials. The epineurium is formed by a looser, more typical collagenous connective tissue. It lies between the fascicles and also covers the surface of the entire nerve. In a peripheral nerve composed of only one fascicle the epineurium immediately surrounds the perineurium.

Slide 18A and 18B: Osmic Nerve Cross Section & Longitudinal Section

Osmium preserves the lipid of the myelin sheaths and stains it black. In the cross section identify the myelin sheaths vs. the pale brown neuronal processes, and note the variation in the thickness of the myelin sheath in different nerve fibers. Also in the cross-section, identify the endoneurium, and the location where perineurium and epineurium would be if they were well preserved. In the longitudinal section, look for nodes of Ranvier. Try to identify a cleft of Schmidt-Lanterman. In the longitudinal section they may be visible as light-staining interruptions of the dark myelin sheath. Make sure you understand the difference between these clefts and the nodes of Ranvier (see Wheater, Fig. 7-7. p.131). Nodes are gaps in the myelin sheath between two Schwann cells, whereas a cleft is found within a single Schwann cell. It represents an area where the
cytoplasm of the Schwann cell persists, i.e., where the cytoplasmic faces of the Schwann cell membrane did not fuse with one another as the myelin sheath formed. As a result, a thin tunnel of cytoplasm is created that spirals through the myelin sheath, connecting the outer collar of Schwann cell cytoplasm (outside the myelin sheath) with the inner collar of Schwann cell cytoplasm (adjacent to the neuronal process). Since the Schwann cell nucleus is located in the outer collar, the clefts are needed to serve as supply lines that keep the inner collar of cytoplasm alive. (Note that the inner and outer collars of Schwann cell cytoplasm can only be seen by electron microscopy).

Slide 4 (HU Box): Artery, Vein & Nerve, Elastic Stain, c.s., and Slide 33 and 33A: Mesenteric Artery and Vein

In Slide 4 locate the nerve. In the human body arteries, nerves and veins often travel together as a neurovascular bundle. In Slides 33 and 33A, locate the many nerves surrounding the mesenteric vessels. These slides illustrate two features that help identify peripheral nerve. The first is that the epineurium surrounding the nerve separates the nerve from surrounding tissues fairly clearly. This layer helps distinguish nerves from collagen fibers or smooth muscle bundles, which lack such a well-defined outer covering. The second characteristic may not be found in the very smallest nerves, but in larger ones the nerve fibers are often quite wavy. Because of this waviness it is not unusual to see cross sectioned nerve fibers side by side with longitudinally sectioned fibers within a single fascicle.

A trichrome stain also helps distinguish nerve from collagen or smooth muscle. Note in slide 33, which is stained with the Masson trichrome, that there is a considerable amount of connective tissue within a peripheral nerve. This is the endoneurium, and it represents thin collagen fibers and reticular fibers. It gives each fascicle a characteristic grayish-blue color, which is easily distinguishable from the darker purple color of smooth muscle or the bright blue-green of large collagen fibers.

Slide 94 (HU Box): Motor End Organs. w.m., Gold Chloride/Formic Acid

This is a "whole mount" (w.m.) specimen rather than sectioned material. It allows us to trace the path of axons as they branch from a peripheral nerve to innervate skeletal muscle fibers at synaptic complexes called neuromuscular junctions. These axons are the processes of the large motor neurons (alpha motor neurons) in the ventral horn of the spinal cord. At each neuromuscular junction the axon forms a disk-like ending called a motor end plate on the surface of the muscle (Wheater, Fig. 7.12, pp. 134-135). Most muscle fibers are innervated by a single motor end plate. A single axon may branch to innervate a few muscle fibers or hundreds of muscle fibers, depending on the muscle. A single axon and all the muscle fibers it innervates make up a motor unit.

IV. SENSORY ENDINGS

Some sensory processes, including those that carry cutaneous pain and temperature sensations, simply end peripherally as free nerve endings. These are normally not visible by light microscopy unless some special staining technique (usually silver staining) has been used. Most other sensory processes end in relation to a sensory receptor. Sensory receptors convert stimuli from the environment into nerve impulses that are carried to the CNS by the afferent (sensory) neurons. Morphologically there is a wide variety of sensory receptor types, not all of which can be assigned a
specific function at this point. We will study three receptors: the Pacinian corpuscle, the Meissner’s corpuscle, and the muscle spindle.

A. Pacinian Corpuscles
   Slide 65 (HU Box): Lamellated (Pacinian) Corpuscle (Pancreas)
   Slide 84 (HU Box): Penis, Fetal, Masson Trichrome
   Slide 43B: Pacinian Corpuscles
   Slide 72: Pancreas - Duodenum, Trichrome

   A Pacinian corpuscle looks like an onion. That is, it is composed of many concentric layers of flattened cells that form a capsule surrounding a cylindrical central region that contains the nerve process (see Wheater, Fig. 7.25, p. 142). Pacinian corpuscles are very large structures and therefore easily located even at low magnification. They are associated with myelinated fibers that lose their myelin sheath after entering the capsule. They can be found near the junction of the dermis and hypodermis of the skin, in the connective tissues of the peritoneum and mesenteries, in ligaments and joint capsules, and in some visceras. They respond to pressure, coarse touch, tension or vibration.

   Be sure to distinguish the Pacinian corpuscle in the pancreas from the islets of Langerhans, which are clusters of endocrine cells in the pancreas.

B. Meissner’s corpuscles
   Slide 43A: Meissner’s Touch Corpuscles

   Meissner’s corpuscles are considerably smaller than Pacinian corpuscles and are located much closer to the body surface. They are found in the dermal papillae immediately beneath the epidermis of the skin, especially in the fingertips, soles of the feet, nipples, eyelids, lips and genitalia (see Wheater, Fig. 7.24a&b, p. 141). Their location is a major aid in identifying Meissner's corpuscles. They are encapsulated by connective tissue and are oval in shape, with one or two neuronal processes at their center (again not visible by H&E). They are oriented with their long axis perpendicular to the surface of the skin. The supporting cells within the capsule often spiral around the nerve so that they are oriented at right angles to the long axis of the corpuscle. Meissner's corpuscles respond to light touch (sometimes called fine touch).

C. Muscle spindles

   Proprioception, a sense of the body's position in space, requires input from pressure, stretch and tension receptors in muscles, joints and tendons. One such receptor in skeletal muscle is the neuromuscular spindle or muscle spindle. Spindles respond to stretching of the muscles, and are essential for the reflex muscle contraction that occurs in response to passive stretch. A muscle spindle is encapsulated by connective tissue and contains several modified skeletal muscle fibers called intrafusal fibers to distinguish them from ordinary skeletal muscle fibers (extrafusal fibers). Intrafusal fibers are much smaller in diameter and paler staining than extrafusal fibers.

Please study muscle spindles in the following slide:

Many of the specimens used for slide 4 also contain a cross-section of a muscle spindle embedded in the connective tissue between skeletal muscle cells. Identify the capsule of the muscle spindle, the small intrafusal muscle fibers and the fluid filled space within the spindle (see Rhodin, Fig. 14-5). Although not evident without special staining, several sensory (afferent) nerve fibers innervate each muscle spindle, wrapping around the intrafusal muscle fibers. When the intrafusal fibers are stretched, these afferent fibers carry impulses to the CNS where they synapse directly on the motor neurons (alpha motor neurons) that innervate the extrafusal fibers of the same muscle, causing them to contract and relieve the stretch on the intrafusal fibers. This pathway is called the spinal stretch reflex, and is in constant use in the adjustment of muscle tone. It also forms the basis for reflex testing such as the knee-jerk test (hitting the quadriceps tendon with the reflex hammer stretches the intrafusal fibers of the muscle, causing the quadriceps to contract and extend the leg at the knee).

Intrafusal muscle fibers also receive their own motor innervation via gamma motor neurons (which are among the smaller neurons in the ventral horn of the spinal cord). Stimulation of gamma motor neurons by descending nerve tracts from the brain causes the intrafusal fibers to contract, thus increasing their sensitivity to stretch.

V. ELECTRON MICROSCOPY (RHODIN)

A. Synapses

A synapse represents a site at which a neuron communicates with another neuron or with an effector cell such as a muscle or gland cell. Synapses may be electrical (in which case they involve the formation of gap junctions between the two cells) or chemical (in which the two cells communicate indirectly via the secretion of neurotransmitters). In humans the vast majority of synapses are chemical. Study the chemical synapses shown in Rhodin Figs. 13-5, 13-6, & 13-11 to 13-14. Distinguish between the presynaptic and post-synaptic sides. Notice the synaptic vesicles and the numerous mitochondria typical of the presynaptic side, and the electron-dense material associated with both membranes, especially the post-synaptic element.

B. Myelin

Myelin is formed by Schwann cells in the PNS, and by oligodendrocytes in the CNS. It consists of multiple layers of tight, spiral wrapping of the plasma membranes of these cells around the nerve process. Axons can be myelinated, as can the peripheral processes of sensory neurons. Dendrites are not myelinated. Study Fig. 13-18 in Rhodin and Figs. 7.5 & 7.6, p. 128-129 in Wheater, which illustrate myelination in the PNS. Identify the nerve process, the myelin sheath, the Schwann cell nucleus, and the outer collar of Schwann cell cytoplasm that surrounds the myelin sheath and contains the Schwann cell nucleus.

Examine the structure of the myelin sheath at higher mag in Fig. 13-19 (this micrograph is from the CNS, but the structure of the myelin sheath itself is the same in both CNS & PNS). As the glial cell membrane wraps around the axon, the cytoplasm is squeezed out and the cytoplasmic faces of the plasma membrane contact one another to form a thick dark-staining line called the major dense line (labeled “major period membrane” in Rhodin). As the extracellular surfaces of the membrane contact one another between successive layers of myelin, they form the thinner, lighter-staining intraperiod or interperiod lines. A small amount of Schwann cell cytoplasm is also found
interior to the myelin sheath, immediately adjacent to the neuronal process. This is the inner collar of cytoplasm (labeled ad-axonal cytoplasm in Rhodin). It is continuous with the outer collar via the spiral tunnel-like passageways formed by clefts of Schmidt-Lanterman.

Study the structure of a node of Ranvier (Figs. 13-22 & 13-24). Notice that near the node the cytoplasmic faces of the Schwann cell membrane fail to fuse, so that Schwann cell cytoplasm remains between them (the perinodal or paranodal Schwann cell cytoplasm). Compare a node of Ranvier to a cleft (incisure) of Schmidt-Lanterman (Figs. 13-20 & 13-23). Recall that a node is a gap between adjacent Schwann cells, while a cleft is contained within a single Schwann cell and represents another region where the cytoplasmic faces of the Schwann cell have not fused.

Compare the myelinated axon of Fig. 13-18 with the unmyelinated axons in Fig. 13-29. Identify the Schwann cell nucleus, Schwann cell cytoplasm and unmyelinated axons in Fig. 13-29. Confirm that each unmyelinated axon is buried within an invagination of the plasma membrane of a Schwann cell, and that multiple axons are associated with a single Schwann cell. This arrangement in the PNS differs from what is observed in the CNS, where unmyelinated axons are not embedded in or otherwise associated with oligodendrocytes.

In Rhodin Fig. 13-28 distinguish between the myelinated and unmyelinated axons in this mixed nerve.

C. Study the following sensory endings:

Muscle spindles (Figs. 14-4 to 14-6). Compare the size of the intrafusal and extrafusal muscle fibers. Identify the capsule (which often has an inner and outer layer separated by a fluid-filled space), and locate the nerve innervating the intrafusal fibers.

Pacinian corpuscles (Figs. 14-9 to 14-10). Observe the multiple layers of flattened cells that make up the capsule. Notice that the afferent neuron ends in a cylindrical region in the center of the corpuscle, and that a corpuscle looks quite different if this cylinder is cut in cross section (Fig. 14-9) or longitudinal section (Fig. 14-10).

D. Study the parts of a typical neuron

Euchromatic nucleus (Figs. 12-3, 13-1, 13-2)
Prominent nucleolus (Figs. 13-1, 13-2)
Perikaryon (Figs. 12-3, 13-1)
Axon (Fig. 12-3; identifiable because it originates from the pale axon hillock and because it does not contain Nissl bodies, even at its proximal end)
Dendrites (Figs. 12-3, 13-1)
Nissl bodies (Figs. 13-1, 13-2, 13-3)
Microtubules (Fig. 13-10) & neurofilaments (Fig. 13-10)

E. Compare the light microscope and electron microscope appearance of:

Multipolar neurons (Figs. 12-3, 12-15 & 13-1). Somatic motor neurons (alpha motor neurons) in the ventral horn of the spinal cord and autonomic neurons (sympathetic and parasympathetic) are examples of multipolar neurons.

Pseudounipolar neurons (Figs. 12-4, & 12-23 to 12-26). The neurons of the dorsal root ganglia (spinal ganglia) are examples of pseudounipolar neurons. If the section does not include their single process, they often appear to lack processes entirely.
F. Compare the neurons of the **dorsal root ganglia** to those of **autonomic ganglia** (Figs. 12-28 to 12-30).

Note that the autonomic neurons tend to be smaller and usually have a more eccentric nucleus. Identify the **satellite cells**, which are found in both types of ganglia (Figs. 12-25 & 26, 12-28 & 12-30). Notice how they completely surround the neuronal soma everywhere except where processes arise from the cell body (Fig. 12-26).

### LABORATORY 7 CHECKLIST

#### NERVE

**LIGHT MICROSCOPY**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Description</th>
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<tbody>
<tr>
<td>dorsal root (spinal) ganglion</td>
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<tr>
<td>pseudounipolar neuron</td>
<td>node of Ranvier</td>
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<tr>
<td>satellite cells</td>
<td>cleft of Schmidt-Lanterman</td>
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<td>Schwann cell</td>
<td>endoneurium</td>
</tr>
<tr>
<td>alpha motor neuron</td>
<td>perineurium</td>
</tr>
<tr>
<td>Nissl bodies</td>
<td>epineurium</td>
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<tr>
<td>axon hillock</td>
<td>neurovascular bundle</td>
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<td>axon</td>
<td>neuromuscular junction on skeletal muscle</td>
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<tr>
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<td>motor unit</td>
</tr>
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<td>Pacinian corpuscle</td>
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<td>Meissner’s corpuscle</td>
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<td>muscle spindle</td>
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<tr>
<td>lipofuscin</td>
<td>intrafusal fibers</td>
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<td>extrafusal fibers</td>
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**ELECTRON MICROGRAPHS**

<table>
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<tr>
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<td>myelin sheath</td>
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<td>satellite cells</td>
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<td>Nissl bodies</td>
</tr>
<tr>
<td>muscle spindle</td>
<td>Pacinian corpuscle</td>
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</table>

**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 7: NERVE

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. Define afferent neuron vs. efferent neuron.
2. What is the difference between a somatic motor neuron and a visceral motor neuron?
3. In the spinal cord, are neuronal cell bodies and glia contained in the gray matter or in the white matter?
4. What is meant by the terms pseudounipolar neuron, bipolar neuron and multipolar neuron? Give an example of each.
5. What is the functional difference between an axon and a dendrite? What are some of their structural differences?
6. What is the correlation between degree of myelination, axon diameter and conduction speed?
7. What is the name for a group of neuronal cell bodies located in the peripheral nervous system (PNS)? In the central nervous system (CNS)?
8. Are there synapses in ganglia?
9. Name several morphological criteria that you could use to distinguish between a dorsal root ganglion and an autonomic ganglion by light microscopy.
10. What are the structural components of a peripheral nerve? Can a single peripheral nerve include sensory fibers as well as motor fibers? Can it include myelinated as well as unmyelinated fibers?
11. Where else can peripheral nerves originate other than from the spinal cord?
12. Which of the connective tissue layers of a peripheral nerve (endoneurium, perineurium, epineurium) directly surrounds each individual fascicle? What specialized function does this layer carry out?
13. Do unmyelinated axons have nodes of Ranvier? Clefts of Schmidt-Lanterman?
14. If you see a peripheral nerve in your section, what other two structures are likely to be running with it?
15. With H&E it is sometimes difficult to distinguish between smooth muscle and peripheral nerves. Name one or two criteria that can help you do this.
16. Define the term “motor unit”.

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17. Name one ultrastructural criterion that can be used to distinguish a neuromuscular junction (motor end plate) on skeletal muscle from an autonomic terminal on smooth muscle.

18. How do the synaptic vesicles in a cholinergic neuron differ morphologically from those in an adrenergic neuron?

19. Suppose you saw a nerve terminal that contained dense-cored synaptic vesicles. Which of the following is this neuron is likely to be: preganglionic sympathetic, preganglionic parasympathetic, postganglionic sympathetic, postganglionic parasympathetic, or a somatic motor neuron ending on skeletal muscle?

20. Name an encapsulated sensory ending that you would be most likely to find in the deep dermis or the hypodermis of the skin. Name one that you would find in the dermal papillae.

21. Name a sensory receptor other than a free nerve ending that you would find within the epidermis.

22. What type of sensory receptor is responsible for the knee-jerk reflex (patellar tendon reflex)? How does tapping the tendon of the muscle cause this reflex?

23. A muscle spindle has a capsule that contains intrafusal muscle fibers. A Golgi tendon organ has a capsule that contains __________.
LABORATORY 8
ENDOCRINE ORGANS
HYPOPHYSIS, THYROID, PARATHYROIDS, & ADRENALS

OBJECTIVES:

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

1. distinguish between hypophysis (pituitary), thyroid, parathyroids and adrenal glands by light microscopy
2. identify acidophils, basophils and chromophobes of the hypophysis by light microscopy
3. distinguish between adenohypophysis and neurohypophysis
4. identify pituicytes, Herring bodies and Rathke’s cysts in the hypophysis
5. distinguish between follicular cells and parafollicular cells of the thyroid by light and electron microscopy
6. describe the organization of a thyroid follicle and the location of parafollicular cells
7. distinguish between chief cells and oxyphils of the parathyroids by light and electron microscopy
8. distinguish between a young and an old parathyroid
9. distinguish between adrenal cortex and adrenal medulla
10. identify the zona glomerulosa, zona fasciculata, zona reticularis, and medulla of the adrenal by light and electron microscopy
11. identify the fetal adrenal cortex (X-zone) & explain its function
12. list the hormonal products and target organs of these endocrine glands

LABORATORY:

Please study the following slides in your set:

I. HYPOPHYSIS (PITUITARY)
   Slide 77 (HU Box): Pituitary Body, and
   Slide 73B or 73C: Pituitary Body (H&E)

   Using low power magnification, identify the two main divisions of the hypophysis, the adenohypophysis and the neurohypophysis. The neurohypophysis includes the pars nervosa and the infundibulum. The adenohypophysis includes the pars distalis, pars tuberalis and pars intermedia. Most of our slides show only pars nervosa, pars distalis; and pars intermedia. Determine which are present on your slides.

   Using higher magnification, note that there are two main cell types in the adenohypophysis, the chromophobes & chromophils. The chromophils can be further classified into acidophils and basophils based on the staining affinities of their secretory
granules. H&E is not particularly good for distinguishing between acidophils and basophils, so you may have difficulty doing this. Certain trichromes do a much better job. The acidophils are the somatotrophs and mammotrophs, while the basophils are the gonadotrophs, thyrotrophs, and corticotrophs. What hormones do the acidophils produce? What ones do the basophils produce? \[Answer: Amongst the acidophils, the somatotrophs produce somatotropin (also called growth hormone), and the mammotrophs produce prolactin. Amongst the basophils, gonadotrophs produce follicle-stimulating hormone (FSH) and luteinizing hormone (LH), thyrotrophs produce thyrotropin (also called thyroid-stimulating hormone or TSH), and corticotrophs produce pro-opiomelanocortin derivatives such as ACTH, lipotropins, endorphins & MSH].

The blood supply to the adenohypophysis has unusual features related to its function. It is supplied by a portal system derived from the superior hypophyseal arteries. A portal system exists if blood goes through two or more separate capillary beds in series before returning to the heart. If the capillary beds are connected by veins it is a venous portal system. If the capillary beds are connected by arteries it is an arterial portal system. In the case of the hypophysis it is a venous portal system. The first capillary bed (primary plexus) is located in the median eminence and infundibulum. It drains into hypophyseal veins, which then break up into the second capillary bed (secondary plexus) located in the pars distalis. Hypothalamic neurons synthesize releasing or inhibiting factors and secrete them into the primary capillary plexus. They are carried to the adenohypophysis where they regulate the secretory activity of specific types of acidophils or basophils. Examine your slides for vessels of the secondary plexus, especially Slide 73B.

Also identify the pars intermedia on Slide 73B. In humans it is quite small and is located near a series of small colloid-filled cysts (Rathke's cysts), which represent the vestiges of Rathke's pouch. Pars intermedia contains mainly basophils and chromophobes. These cell types may extend for a short distance into the pars nervosa.

The neurohypophysis looks much less cellular and more fibrous than the adenohypophysis. The structures that appear to be fibers are really the axons of neurons whose cell bodies are located in the hypothalamus. They form the hypothalamic-hypophyseal tract. These neurons synthesize the peptide hormones oxytocin and antidiuretic hormone (ADH, also called vasopressin) in their cell bodies in the hypothalamus, package them in secretory granules along with proteins called neurophysins, transport them down their axons into the pars nervosa and release them there. Groups of secretory granules can form dilations called Herring bodies along the length of the axon near its terminal. These pale-staining, granular structures will probably not be visible in your sections but can be seen more easily by EM. Oxytocin and vasopressin are picked up by the capillary bed of the neurohypophysis, which is derived mainly from the inferior hypophyseal arteries, and carried out of the pituitary. The nuclei visible in the pars nervosa do not belong to neurons, rather most belong to pituicytes. Pituicytes are believed to be glial in nature.

II. THYROID AND PARATHYROID

Slide 78 (HU Box): Parathyroid, Primate (Thyroid, Parathyroid), or Slide 75B: Parathyroid Gland

Most versions of both these slides contain portions of the thyroid as well as parathyroid. Using the low power objective, it is apparent that the thyroid consists of spherical follicles, which represent the structural and functional unit of the gland,
whereas in the parathyroid the cells are arranged in long cords or clusters, and follicles are rare or absent.

The parathyroid contains two types of parenchymal cells: chief cells (also called principal cells) and oxyphils. Oxyphils are not present on most of our slides, and you will probably have to consult the videodisk or the Virtual Microscopy slides on the course website for good examples. They tend to increase in number with increasing age, so the explanation for their scarcity here may be that this tissue on our slides was taken from newborns.

Study the chief cells at higher magnification. They have a central round nucleus and a small amount of cytoplasm. Some contain large amounts of glycogen, which is often lost during tissue processing, making their cytoplasm appear pale. They secrete parathyroid hormone or PTH, which raises serum calcium levels by stimulating bone resorption, absorption of dietary calcium from the GI tract, and resorption of calcium from the urinary filtrate.

Oxyphils tend to occur in groups rather than as scattered individual cells. They are larger than principal cells, but have a slightly smaller, darker nucleus (See Rhodin, Fig. 22-2, p. 256). Therefore a region containing oxyphils will have fewer nuclei per unit area than a region containing chief cells. The cytoplasm of the oxyphils is more acidophilic than that of the chief cells due to the presence of many mitochondria.

Slide 78 (HU Box): Parathyroid, Primate (Thyroid, Parathyroid), or Slide 74 (Several Versions): Thyroid

Each thyroid follicle consists of a lumen containing an eosinophilic material called colloid, surrounded by a single layer of epithelial cells (follicular cells). Note the abundant capillaries in the sparse connective tissue between follicles. The height of the follicular cells increases with increasing functional activity of the individual follicle. For reasons that are unclear, it is not unusual to find a follicle with almost squamous follicular cells that is located immediately adjacent to a follicle with much taller cells.

Examine the cells of the thyroid using higher magnification. The follicular cells all rest on the basement membrane and reach all the way to the lumen. They synthesize the protein called thyroglobulin, secrete it into the lumen, iodinate it, and carry out the coupling reaction that converts some of the tyrosine moieties to T3 & T4 residues in the thyroglobulin molecule. They then take up the iodinated thyroglobulin into phagosomes (called “colloid droplets”) that may be visible by LM. These fuse with lysosomes to form secondary lysosomes where the thyroglobulin is hydrolyzed to release active T3 & T4 molecules along with the other amino acids. When the follicular cells are highly active the edge of the colloid in the lumen may appear scalloped, indicating regions where colloid has been taken up into the follicular cell by endocytosis.

Thyroid tissue is difficult to fix and to section. In some slides you may see a sectioning artifact called "chatter" in the colloid. Chatter looks like parallel light and dark staining bands that represent areas where the section is thick (dark band) next to areas where it is much thinner (light band). This is caused by the bouncing of the knife as it cuts through the colloid, which is a very hard material once it is fixed. Thyroid may also show shrinkage artifacts, which create artificial spaces where none exist in life. For example, the follicles may be separated from one another by artifically large spaces, or there may be a uniformly wide gap between the colloid and the follicular cells.

Another thyroid cell type is the parafollicular cell (C cell). Their cytoplasm is pale-staining (the designation C cell originally referred to their pale or "clear" appearance) and they are larger than follicular cells. Parafollicular cells are quite rare in humans and you will probably not find any on your slides. If you don't, consult the videodisk for good
examples. Parafollicular cells can be part of the follicular epithelium, in which case they rest on the basement membrane but do not reach the follicular lumen. They can also occur in clusters in the connective tissue between neighboring follicles. Care must be taken not to confuse a tangential section through a follicle with an interfollicular nest of C cells. What hormone is produced by the parafollicular cell? (Answer: *They produce the protein hormone calcitonin. So it is handy to associate these “C” cells with their product, calcitonin.*) What is its effect? (Answer: It limits bone resorption by inhibiting the action of osteoclasts, and is thus antagonistic to the effect of PTH on bone). Do parafollicular cells secrete their product into the follicular lumen? (Answer: No, they secrete across the epithelial basement membrane into the connective tissue between follicles, where the calcitonin is picked up by the fenestrated capillaries.) Are parafollicular cells essential for life? (Answer: No, because calcitonin is not essential for life. This is in contrast to PTH from the parathyroids, which is required.)

III. ADRENAL (SUPRARENAL) GLAND

Slide 79 (HU Box): Suprarenal Gland, Human, or Slide 77 or 78: Adrenal

The adrenal has a connective tissue capsule surrounding the cortex and the medulla. The outer region is the cortex (which is divided into three distinct zones), while the medulla is centrally located and structurally more disorganized.

The three zones of the cortex can be distinguished by the morphology of the cells and their arrangement. The outermost is the zona glomerulosa, which has relatively small cells arranged in spherical clusters or short curved cords. The cells of the zona fasciculata are larger and paler, and are sometimes referred to as "spongiocytes" because their cytoplasm contains many lipid droplets that are often extracted during tissue processing, making the cytoplasm appear vacuolated or spongy. The cells of the zona fasciculata are arranged in long straight cords (fascicles) that run radially. Numerous sinusoidal capillaries run parallel to the cords. The innermost zone of the cortex is the zona reticularis. Here the cells are less vacuolated (hence their cytoplasm looks darker-staining) and they are arranged in irregular anastomosing cords. What hormones are secreted in each zone? (Answer: The zona glomerulosa produces mineralocorticoids such as aldosterone. The zona fasciculata produces glucocorticoids such as corticosterone. The zona reticularis produces weak androgens such as dehydroepiandrosterone and small amounts of glucocorticoids.)

The medulla contains larger cells than the cortex. They are called chromaffin cells and are generally arranged less regularly than the cells of the cortex, giving the medulla a “messier” appearance. What hormones do chromaffin cells produce? (Answer: They produce either epinephrine or norepinephrine. In H&E stained sections it is usually not possible to distinguish between them). Occasional neuronal cell bodies can also be found in the medulla. They probably innervate blood vessels. Review the medulla's innervation and blood supply. Note the large veins present in the medulla. These unite to form a single adrenal vein. Each adrenal receives blood from three separate arteries but is drained by only one vein.

IV. ELECTRON MICROSCOPY (RHODIN)

A. HYPOPHYSIS
Note the membrane-bounded secretory granules in the chromophils of the adenohypophysis (Figs. 20-4 & 20-55). Although it is not necessary to be able to do so, nevertheless you should realize that it is possible to distinguish between the various types of acidophils and basophils based on the size, morphology and distribution pattern of the secretory granules in the cytoplasm. Find cells that contain few granules. These are probably chromophobes. Identify the capillaries of the adenohypophysis and realize that these are part of the secondary capillary bed in the venous portal system that supplies the adenohypophysis. (See Rhodin, Fig. 20-1 for a diagram).

In the pars nervosa identify the Herring bodies (Figs. 20-16 & 20-18), and confirm that they contain large numbers of secretory vacuoles. What products are contained in these vacuoles? (Answer: Oxytocin and ADH along with neurophysins). Note that most of the nuclei in the pars nervosa belong to pituicytes. Where are the cell bodies of the neurons whose axons contain the Herring bodies? (Answer: In the hypothalamus).

B. THYROID

Identify the thyroid follicular cells (Figs. 21-3 & 21-4). Their numerous small lysosomes (Fig. 21-5) are located in the basal cytoplasm of inactive cells, but migrate to the apical cytoplasm in the active cell and then fuse with colloid droplets (Fig. 21-4). Rhodin calls these colloid droplets secretory granules (Fig. 21-4), but they are actually phagosomes that have taken up iodinated colloid from the lumen. The noniodinated thyroglobulin is secreted from the cells into the follicular lumen in small inconspicuous clear vesicles. Note the apical microvilli. They too increase in number in active cells. What important enzyme is thought to be associated with the apical plasma membrane of follicular cells? (Answer: Thyroid peroxidase). What is its function? (Answer: It oxidizes iodide so that it can be used to iodinate thyroglobulin, it catalyzes the iodination reaction, and it also catalyzes the coupling reaction that converts some of the tyrosine residues of thyroglobulin to T3 & T4.) The follicular cell also has a reasonable amount of rough endoplasmic reticulum (RER), which is the site of synthesis of thyroglobulin. Active follicular cells can be identified on the basis of their RER, since they are one of the few cell types in which the RER cisternae are normally swollen or distended.

Observe the location and morphology of the parafollicular cells (C cells) (Figs. 21-2 & 21-6). Even by EM their cytoplasm usually appears paler than that of follicular cells. Notice that the secretory granules are more numerous at the basal end of the cell, since the C cell secretes across its basal plasma membrane into the connective tissue between follicles. The hormone is then picked up by the fenestrated capillaries (Fig. 21-6). This very permeable type of capillary is typical of endocrine glands.

C. PARATHYROIDS

Identify the chief cells (principal cells) and oxyphils (Fig. 22-4). Notice that some chief cells contain considerable amounts of glycogen (Figs. 22-3 & 22-4). The chief cells shown in Rhodin contain very few secretory granules (Fig. 22-4), suggesting that there was a high demand for PTH at the time the tissue was fixed, causing the hormone to be secreted soon after its synthesis rather than being stored in cytoplasmic vacuoles.

The major ultrastructural feature of the oxyphil is large number of mitochondria (Figs. 22-4 & 22-7). Recall that the function of these cells is unknown, and that they increase in number with age.

Notice the adipocytes (Figs. 22-2 & 22-3). In the parathyroid, adipocytes also increase in number with increasing age.
D. ADRENAL

Study the arrangement of the cells in the zona glomerulosa (clusters or arched cords), in the zona fasciculata (straight cords running toward the medulla), and in the zona reticularis (irregular anastomosing cords) (Figs. 23-3 & 23-5). Identify the sinusoidal capillaries between cords. Note the abundant lipid droplets in the cells (spongiocytes) of the zona fasciculata (Fig. 23-3). The cortical cells produce steroids. What three ultrastructural features are characteristic of steroid-secreting cells (Figs. 23-6 & 23-7)? (Answer: Mitochondria with tubular cristae, abundant SER, and many lipid droplets). In what way is the morphology of the cells of the zona glomerulosa unusual for steroid-secreting cells? (Answer: Their mitochondria have the usual shelf-like cristae rather than tubular cristae.)

Compare the epinephrine-producing and the norepinephrine-producing chromaffin cells of the medulla (Figs. 23-11 to 23-14). Observe that it is possible to tell these cells types apart based on the size and morphology of their secretory granules (Figs. 23-13 & 23-14). Norepinephrine producers have larger granules that contain an irregular, highly electron-dense core that is often eccentrically placed within the granule. Epinephrine producers have smaller granules with lighter staining cores that are usually centrally placed, leaving a more uniform halo between the membrane of the granule and the core.
# LABORATORY 8 CHECKLIST

## ENDOCRINE ORGANS

### LIGHT MICROSCOPY

<table>
<thead>
<tr>
<th>HYPOPHYSIS</th>
<th>THYROID</th>
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<tbody>
<tr>
<td>adenohypophysis</td>
<td>follicle</td>
</tr>
<tr>
<td>pars intermedia</td>
<td>follicular cell</td>
</tr>
<tr>
<td>Rathke’s cysts</td>
<td>colloid</td>
</tr>
<tr>
<td>chromophobe</td>
<td>parafollicular (C) cell</td>
</tr>
<tr>
<td>acidophil</td>
<td>ADRENALS</td>
</tr>
<tr>
<td>basophil</td>
<td>cortex</td>
</tr>
<tr>
<td>neurohypophysis</td>
<td>zona glomerulosa</td>
</tr>
<tr>
<td>Herring bodies</td>
<td>zona fasciculata</td>
</tr>
<tr>
<td>pituicytes</td>
<td>zona reticularis</td>
</tr>
<tr>
<td>PARATHYROID</td>
<td>medulla</td>
</tr>
<tr>
<td>chief (principal) cell</td>
<td>chromaffin cells</td>
</tr>
<tr>
<td>oxyphil</td>
<td></td>
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</table>

### ELECTRON MICROGRAPHS

<table>
<thead>
<tr>
<th>HYPOPHYSIS</th>
<th>THYROID</th>
</tr>
</thead>
<tbody>
<tr>
<td>pars nervosa</td>
<td>follicle and follicular cell</td>
</tr>
<tr>
<td>Herring bodies</td>
<td>parafollicular (C) cell</td>
</tr>
<tr>
<td>adenohypophysis</td>
<td>colloid</td>
</tr>
<tr>
<td>ADRENALS</td>
<td>PARATHYROID</td>
</tr>
<tr>
<td>mitochondria with shelf-like cristae</td>
<td>chief (principal) cell</td>
</tr>
<tr>
<td>mitochondria with tubular cristae</td>
<td>oxyphil</td>
</tr>
</tbody>
</table>

**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 8: ENDOCRINE ORGANS

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. What is the embryological origin of the adenohypophysis? Of the neurohypophysis?
2. What is the definition of a portal system? Does the pituitary have a venous or arterial portal system?
3. What are the functions of the portal system that is found in the pituitary?
4. Trace the life history of a molecule of oxytocin. Where is it synthesized? Where is it secreted? What are its target organs? What effects does it have on these target organs?
5. Do males secrete oxytocin?
6. Is antidiuretic hormone synthesized in the pituitary? What is its major target organ and what effects does it have there?
7. What are Herring bodies? Where do you find them?
8. If the pituitary stalk were cut, which type of acidophil or basophil in the adenohypophysis would increase its production of secretory product? Why?
9. Why does the presence of numerous mitochondria make the cytoplasm of a cell eosinophilic? What endocrine gland cell contains extremely large numbers of mitochondria, yet has no known function?
10. Explain why thyroid tissue can sometimes be found within the tongue.
11. The thyroid follicles can sometimes be confused with the lactating mammary gland. How can you distinguish between the two by LM?
12. What membrane junctions are found between thyroid follicular cells? Why are they important to the functioning of the organ?
13. Active thyroid follicular cells and aging plasma cells tend to develop irregular, distended cisternae of RER. If you had an EM of a cell that showed these swollen RER cisternae, how would you decide if it were a follicular cell or a plasma cell?
14. Chromaffin cells of the adrenal medulla can produce either epinephrine or norepinephrine. How do the cells of the adrenal cortex determine which hormone a particular chromaffin cell will produce?
15. How are mitochondria, SER and lipid droplets involved in steroid synthesis?
LABORATORY 9
CARTILAGE & BONE

I. CARTILAGE

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

1. identify the following features of cartilage:
   - chondroblasts
   - chondrocytes
   - perichondrium (fibrous layer & cellular layer)
   - lacunae
   - isogenous groups
   - territorial matrix
   - interterritorial matrix

2. describe and identify by light and electron microscopy the three types of cartilage:
   - hyaline cartilage
   - elastic cartilage
   - fibrocartilage

3. give examples of locations in the body where each type of cartilage is normally found

4. appreciate that cartilage grows by appositional and interstitial growth

LABORATORY:

Cartilage is one of the specialized types of connective tissues. During this laboratory, you should study the structural features of cartilage and relate the features to the function, growth and nutrition of the tissue.

Please study the following slides in your set:

A. Hyaline Cartilage
   Slide 35 (HU Box): Hyaline Cartilage, Human (Trachea)
   Slide 70 (HU Box): Trachea and Esophagus, c.s., or Slide 46A or 46B: Trachea

Hyaline cartilage is the most common type in the body. By light microscopy it is characterized by a matrix that appears clear and glassy (hyaline means glassy), i.e., no obvious fibers are visible within the matrix. This is due to the fact that hyaline cartilage contains collagen type II, which does not form large fibers.

In the trachea, the hyaline cartilage is present as a series of C-shaped rings. Identify the chondrocytes, which reside in spaces called lacunae within the cartilage matrix. Note the characteristic translucent appearance of the matrix of hyaline cartilage. The matrix immediately surrounding the lacuna has the highest concentration of
proteoglycans. It is referred to as the territorial matrix. If the proteoglycans have been preserved during fixation, the territorial matrix will stain darkly and will be basophilic. The matrix further from the lacunae is the interterritorial matrix, which is lighter staining due to a lower proteoglycan content. Recall that proteoglycans are often lost during tissue fixation. If this occurs, the territorial and interterritorial matrices will be eosinophilic due to the now unmasked presence of collagen, and will be quite similar to one another in staining intensity.

Identify an isogenous group, which is a cluster of chondrocytes either occupying a single lacuna or occupying lacunae that are unusually close together. Isogenous groups are presumed to have arisen by mitosis of a single precursor chondrocyte. They are therefore more common in rapidly growing cartilage. The close proximity of the cells in an isogenous group suggests that not much time has elapsed since mitosis, and therefore not much matrix has been laid down between them.

Hyaline cartilage is surrounded by a perichondrium except at the articular surfaces of joints. The perichondrium has an outer fibrous layer and an inner chondrogenic or cellular layer. Chondroblasts differentiate from the cells of the chondrogenic layer. By LM the chondrogenic and fibrous layers are only easily distinguishable during periods when active appositional growth is occurring.

B. Elastic Cartilage
Slide 37 (HU Box): Elastic Cartilage, Elastic Stain, or Slide 4A or 4B: External Ear

The characteristic feature of elastic cartilage is the presence of elastic fibers, which are visible in the matrix by LM. With the special elastic stain used on slide 4B, they are easily visible as fine purple fibers. They are less easy to find in H&E preparations, but with careful observation they can be seen (slide 4A). Elastic cartilage also contains type II collagen fibers in the matrix, but again these are so thin that they are normally not visible by LM. Observe the chondrocytes, lacunae, and perichondrium of elastic cartilage.

Slide 18 (HU Box): Epiglottis, l.s.
This is another example of elastic cartilage. Some sections show degeneration of the cartilage and replacement by adipose tissue and glands. Such degeneration is presumably age-related.

C. Fibrocartilage
Slide 36 (HU Box): Fibrocartilage
Slide 45 (U Michigan Collection): Intervertebral Disk (See virtual microscope)

Fibrocartilage has a distinctive matrix containing type I collagen fibers arranged in bundles that are thick enough to be visible by LM even in H&E stained sections. The chondrocytes are sometimes arranged in characteristic rows, and may be surrounded by a thin rim of less fibrous matrix that contains type II collagen fibers. Fibrocartilage usually lacks a perichondrium.

Fibrocartilage is fairly rare in humans. It is found where tough connective tissue joins cartilage or bone, i.e., at the insertions of tendons or ligaments, in intervertebral disks, etc. Slide 45 (U. Michigan) contains very clear areas of fibrocartilage within the annulus fibrosus of the intervertebral disk. The fibrocartilage of the intervertebral disk has a characteristic lamellar (layered) pattern, with the chondrocytes ordered in rows.
Slide 36 is from the pubic symphysis. Look for fibrocartilage in the joint between the two pubic bones. Some of these slides contain only fibrocartilage in the symphysis, while others have only hyaline cartilage, and still others have both hyaline and fibrocartilage that grade into one another.

Note: Some versions of Slide 8A or 8B contain a region of muscle insertion. If you are very fortunate you may see the following transition of tissues at the insertion point: skeletal muscle, tendon, fibrocartilage, bone. Fibrocartilage is often present in small amounts between a tendon or ligament and a bone.

II. BONE

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

1. distinguish between demineralized bone sections and ground bone
2. identify the following features of bone by light microscopy:
   - Haversian system (secondary osteon) including:
     - Haversian canal (central canal)
     - lacunae
     - canaliculi
   - interstitial lamellae
   - inner circumferential lamellae
   - outer circumferential lamellae
   - Volkmann’s canal (perforating canal)
   - periosteum
   - endosteum
   - osteoid
   - bone spicules and trabeculae
   - Howship’s lacunae (resorption lacunae)
3. identify the following cell types by light and electron microscopy:
   - osteoblast
   - osteocyte
   - osteoclast
4. distinguish between the structure of:
   - woven bone vs. lamellar bone
   - compact bone vs. cancellous bone
5. differentiate a resorptive surface from a depository surface of a bone based on the cell types that are present
6. describe the blood and nerve supply to bone
7. appreciate the difference between bone (the tissue) and a bone (an organ including bone tissue, marrow, ordinary connective tissue, blood vessels, and in most cases articular cartilage)
8. understand that bone tissue increases in mass only by appositional growth
LABORATORY:

Bone is too hard to be embedded and cut by routine methods unless it has first been demineralized (decalcified). If, however, the mineralized matrix is left intact, then instead of being sectioned with a microtome, bone must be ground down to make it thin enough for light to pass through it. In ground sections, the mineral of the matrix has been preserved but its organic components have been destroyed along with the cells. To gain a realistic understanding of bone you must examine both ground bone and demineralized sections.

In this laboratory, we will focus first on the appearance of lamellar bone by looking at bone tissue taken from adult samples, using ground sections and decalcified sections. Then, we will look at bone from growing individuals to differentiate lamellar bone from primary tissues such as woven bone.

Slide 38 (HU Box): Bone, Ground, Unstained, or
Slide 6: Ground Bone

These ground sections contain mature (lamellar) compact bone. Most of the cortex is composed of osteons (Haversian Systems) that are being viewed in cross section. If the ground section has been stained with India ink, most of the spaces in the matrix should have filled with the black ink, making them easily identifiable. Find the following spaces: the central canal (Haversian canal) of an osteon, perforating canals (also called Volkmann’s canals), lacunae, and canaliculi. In life, the Haversian canals and Volkmann’s canals contained blood vessels, connective tissue, nerve and endosteum. Lacunae contained only osteocyte cell bodies. Canaliculi contained long cytoplasmic processes that extend out from each osteocyte and contact the processes of neighboring osteocytes. Observe that an osteon consists of layers called concentric lamellae that surround the central canal. All the lacunae within an osteon are interconnected by canaliculi, but there is little or no direct interconnection between the canaliculi of neighboring osteons. Appreciate that Haversian canals and Volkmann’s canals differ from one another in orientation: Haversian canals run parallel to the long axis of long bones, while Volkmann’s canals run at an angle to the long axis, and serve to interconnect Haversian canals and also to link them to the blood supply in the periosteum and the marrow cavity. Identify the cement line that surrounds each osteon, and any interstitial lamellae that may lie between osteons. The latter represent areas of lamellar bone (old osteons, circumferential lamellae, etc.) that were incompletely removed during bone remodeling. See if your section has any circumferential lamellae. These are lamellar sheets of bone that encircle either the outer (periosteal) surface of compact bone (outer circumferential lamellae), or the inner (endosteal) surface (inner circumferential lamellae), in direct contact with the marrow cavity.

Slide 50 (U. Michigan Collection): Femur, Monkey (See Virtual Slidebox)
Slide 7 or 7A: Rib

Slide 50 (U. Michigan) is a cross section through the shaft of an adult femur that has been demineralized in acid or a chelating agent and then sectioned on a microtome. In demineralized sections the mineral component of the matrix is lost, but the organic component (mostly collagen) is preserved along with the cells. The section contains entirely cortical (compact) bone. Identify the same features you observed in the slides of ground bone (secondary osteons, Haversian canals, Volkmann’s canals, lacunae, interstitial lamellae, circumferential lamellae) and compare their appearance in the two types of preparations. Notice particularly that the lacunae contain osteocytes within them, as the cells are preserved in this preparation. Osteoblasts and osteoclasts are not seen well in this section.
Slide 7 is a cross-section through the Human Rib. The rib has a very thin outer shell of cortical (compact) bone, and is filled with a marrow cavity containing bone marrow and spongy bone (cancellous bone). Red marrow contains stem cells and the developing red and white blood cells. In older bone, red marrow is replaced in most locations by yellow marrow containing mostly adipocytes. Find an area towards the center of the marrow cavity and study the trabeculae. Trabeculae are thin enough that they contain no blood vessels. The osteocytes within a trabecula are nourished by blood vessels in the marrow cavity. An area with many trabeculae is a region of cancellous or spongy bone. Examine the marrow nearby. Contrast this spongy bone with the more solid looking regions of compact bone.

Slide 48 (U. Michigan Collection): Fetal Leg (See Virtual Slidebox)

These sections were taken from bones that were still actively growing. Slide 48 (U. Michigan) is a section through a fetal leg (tibia and fibula). At this stage of development, the bone is almost exclusively woven in organization. Contrast the organization of the woven bone tissue (e.g. large, numerous and irregularly spaced osteocytes, random arrangement of collagen fibers in the matrix) to the lamellar bone you examined earlier. Notice that the bone is very porous. As development continues, these vascular and CT filled spaces will be filled in with lamellar bone, consolidating it into a region of cortical bone, and the middle of the bone will be hollowed out to form the marrow cavity (hematopoietic marrow can already be seen in the center of this section). Notice some areas of bluish, acellular tissue towards the center of the bone section. This is calcified cartilage - a remnant of the endochondral ossification process that you will learn about before the next laboratory.

Slide 98HU contains mostly or exclusively cortical bone. However, much of the bone tissue is non-lamellar, and within the lamellar bone regions, relatively few well-developed Haversian systems are seen. This suggests that this bone was young and had not undergone significant remodeling.

Use these slides to study the periosteum and endosteum. These are best studied in a growing bone because they will contain a clear osteogenic layer. Periosteum is the connective tissue and cell layer covering the outer surface of a bone everywhere except where there is articular cartilage. Analogous to the perichondrium, it has an outer fibrous layer (connective tissue) and an inner more cellular layer called the osteogenic layer. The osteogenic layer contains osteoprogenitor cells that differentiate into osteoblasts. Endosteum is the thinner layer that lines the marrow cavity (including the surface of any bony trabeculae), central canals, and perforating (Volkmann’s) canals. It is sometimes composed only of flat, inconspicuous osteoprogenitor cells. At other times these differentiate into larger more easily identifiable osteoblasts (see below).

Use these slides to study the various bone cells as well:

1) Osteoblasts are the intensely basophilic uninucleate cells located on any surface of bone of bone tissue where new bone is being laid down. The basophilia is due to large amounts of RER that is producing components of bone matrix such as collagen. Osteoblasts are in direct contact with the bone matrix, and often line up next to one another to form a layer of cells that resembles a simple cuboidal or columnar epithelium. Osteoblasts can be found at the periosteal surface and also at endosteal surfaces of bone.
2) Osteocytes are derived from osteoblasts that have become completely surrounded by the matrix they have produced. Each osteocyte occupies a space within the matrix called a lacuna. Each lacuna is surrounded by a thin rim of osteoid, which is the incompletely calcified matrix of bone. Osteoid may stain more eosinophilic than the completely calcified regions of the matrix. Sometimes the osteoid layer is so thin that it is only visible by electron microscopy. Thin cytoplasmic processes extend from each osteocyte into narrow tunnels within the matrix called canaliculi. Canaliculi are usually more evident in ground bone than in demineralized sections. The cell processes of neighboring osteocytes communicate with one another within the canaliculi.

3) Osteoclasts are very large multinucleated cells that are bone marrow derived. They have a bone marrow precursor in common with monocytes (the GM-CFU or granulocyte-monocyte colony forming unit, which you will study later as part of hematopoiesis). Osteoclasts have an eosinophilic cytoplasm. They are the cell type responsible for resorbing bone matrix during bone growth and remodeling. They can be found on any surface of bone (periosteal or endosteal) where bone resorption is occurring. By removing bone matrix, the osteoclast produces and occupies a depression in the surface of the bone matrix called a Howship's lacuna (resorption lacuna).

III. ELECTRON MICROSCOPY (RHODIN)

A. CARTILAGE

Compare the LM and EM views of the three types of cartilage:

- Hyaline cartilage (Figs. 8-1 to 8-3)
- Elastic cartilage (Figs. 8-7 to 8-9)
- Fibrocartilage (Figs. 8-11 to 8-13)

Note that one way to distinguish between them at the EM level is to look at the fibers and fibrils in the matrix. The matrix of hyaline cartilage contains very thin collagen fibrils typical of type II collagen (Fig. 8-3), the matrix of elastic cartilage contains elastic fibers (#6 in Fig. 8-9), and the matrix of fibrocartilage contains the thick fibrils and fibers of type I collagen (Fig. 8-13).

Study the general organization and development of hyaline cartilage (Fig. 8-2). Identify the perichondrium with its fibrous layer (#1&2 in Fig 8-2) and chondrogenic layer (#3), chondroblasts (#4), and chondrocytes (#5). Identify the isogenous group near the center of the micrograph.

Observe the characteristics of a typical mature chondrocyte (Figs. 8-3, 8-9 & 8-13): sparse short microvilli, relatively euchromatic nucleus, and moderate amounts of Golgi and RER. Mature chondrocytes also often contain lipid droplets (Figs. 8-2, 8-3 & 8-8) and/or abundant glycogen (Fig. 8-3). Extraction of these components during fixation is one reason chondrocyte cytoplasm often looks pale by LM.

B. BONE

Bone preparations for EM are usually demineralized rather than ground. However, decalcification is rarely 100% effective, so that "demineralized" bone matrix almost always still contains some dark crystals of inorganic salts (Figs. 9-11 and 9-14), which help to distinguish bone from cartilage. This material is mainly hydroxyapatite.

Observe the uncalcified region of the matrix (osteoid) immediately surrounding osteocytes (Fig. 9-11). It contains collagen fibrils, but no inorganic salt crystals.
Be able to distinguish the three types of bone cells from one another and from chondrocytes:

**Osteocytes** (Figs. 9-10 & 9-11): Osteocytes are by definition completely surrounded by bone matrix. Note the long thin cytoplasmic processes, which extend into canaliculi. Chondrocytes may have short microvilli, but would never have the long processes seen in osteocytes. Although not illustrated in these micrographs, the cytoplasmic processes of neighboring cells contact each other via gap junctions.

**Osteoblasts** (Fig. 9-14): By definition, an osteoblast is actively secreting the components of bone matrix, but is not yet surrounded on all sides by matrix. Osteoblasts have more RER and Golgi than osteocytes, since the latter are synthetically less active cells.

**Osteoclast** (Figs. 9-18 to 9-21): Observe the much larger size of osteoclasts vs. osteoblasts or osteocytes (Fig. 9-18). Identify the ruffled border (Figs. 9-19 to 9-21). Although not well illustrated in these micrographs, be aware that osteoclasts are multinucleate and that they contain large numbers of lysosomes.

Study the ultrastructure of a Haversian canal and identify the endosteum (#5 in Fig. 9-22). What else can the canals contain in addition to blood vessels and endosteum? *(Answer: Reticular fibers, connective tissue cells and nerves).*

Identify the two regions of the periosteum (Fig. 10-5): the outer connective tissue (fibrous) layer with many collagen fibers (#2) and fibroblasts (#1), and the inner osteogenic layer with osteoprogenitor cells (#3) and less collagen. Observe the differentiation of osteoblasts (#4) into osteocytes (#8).
### LIGHT MICROSCOPY

<table>
<thead>
<tr>
<th>BONE</th>
<th>CARTILAGE</th>
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<tbody>
<tr>
<td>osteocyte</td>
<td>osteocyte</td>
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<tr>
<td>osteon</td>
<td>osteoclast</td>
</tr>
<tr>
<td>Haversian (central) canal</td>
<td>Howship’s lacuna</td>
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<tr>
<td>Volkmann’s (perforating) canal</td>
<td>woven bone</td>
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<tr>
<td>lacuna</td>
<td>spongy bone</td>
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<tr>
<td>canaliculus</td>
<td>CARTILAGE</td>
</tr>
<tr>
<td>interstitial lamellae</td>
<td>hyaline cartilage</td>
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<tr>
<td>cement lines</td>
<td>elastic cartilage</td>
</tr>
<tr>
<td>concentric lamellae of an osteon</td>
<td>fibrocartilage</td>
</tr>
<tr>
<td>circumferential lamellae</td>
<td>chondrocyte</td>
</tr>
<tr>
<td>periosteum</td>
<td>lacuna</td>
</tr>
<tr>
<td>endosteum</td>
<td>territorial matrix</td>
</tr>
<tr>
<td>marrow cavity</td>
<td>isogenous group</td>
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<tr>
<td>osteoblast</td>
<td>perichondrium</td>
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### ELECTRON MICROGRAPHS

<table>
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<tr>
<th>BONE</th>
<th>CARTILAGE</th>
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<tbody>
<tr>
<td>osteocyte</td>
<td>hyaline cartilage</td>
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<td>osteoblast</td>
<td>elastic cartilage</td>
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<td>osteoclast</td>
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<td>perichondrium</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 9: CARTILAGE & BONE

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 proteoglycans cause the matrix of cartilage to stain basophilic?
2. Why do chondroblasts have more RER and Golgi cisternae than mature chondrocytes?
3. Why don’t you see large secretory granules in osteoblast cytoplasm?
4. What is the major type of collagen found in the matrix of hyaline cartilage? Of elastic cartilage? Of fibrocartilage?
5. Why do you think articular hyaline cartilage does not repair as well as hyaline cartilage in other locations?
6. Which of the following surfaces in bone tissue is/are lined or covered by endosteum: Outer circumferential lamellae, inner circumferential lamellae, Haversian canal, Volkmann’s canal, trabeculae, lacunae?
7. Why can’t bone grow interstitially?
8. What is the difference between bone tissue and the organs known as bones?
9. What is the difference between woven bone and lamellar bone?
10. What is the difference between spongy bone and compact bone?
11. Is it true that some bones are composed completely of compact bone and others are composed completely of spongy bone?
12. Can spongy bone be woven bone? Can it be lamellar bone?
13. Can compact bone be woven bone? Can it be lamellar?
14. What cells give rise to osteoclasts?
15. What purpose does the ruffled membrane serve in osteoclasts? Is it found over the entire surface of an osteoclast?
LABORATORY 10
BONE FORMATION & JOINTS

I. BONE FORMATION & JOINTS

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

1. describe the two methods of bone formation:
   intramembranous bone formation
   endochondral bone formation

2. recognize the following in a developing long bone:
   diaphysis
   epiphysis
   periosteal bone collar
   primary ossification center
   secondary ossification center
   marrow cavity
   calcified cartilage
   woven bone

3. understand the histology of bone growth in length and in width

4. identify the zones of cartilage found in the epiphyseal growth plate during endochondral ossification

5. Demonstrate examples of bone modeling and remodeling processes using histological sections.

6. describe the histology of synovial joints, including articular cartilage

LABORATORY:

Please study the following slides in your set:

A. Intramembranous Bone Formation
   Slide 5 (HU Box): Developing Membrane Bone, Human Fetus
   Slide 39 (HU Box): Membrane Bone, Fetal Skull, Coronal Section, Mallory Trichrome
   Slide 58: Development of Tooth
   Slide 58A: Tooth Formation, or
   Slide 58B: Tooth, Development, Late Stages

Intramembranous bone formation occurs when bone forms directly in mesenchyme rather than by replacing a cartilage model of the bone. Much of intramembranous bone formation in humans occurs in the skull.

Slide 5 (HU Box) is an example of intramembranous ossification occurring in one of the flat bones of the cranial vault. On the slides of tooth development (39 HU, 58, 58A or 58B) find the developing mandible or maxilla. The tooth itself is not what you want to study now. Observe the irregular individual spicules of bone tissue and the osteocytes in lacunae within the spicules. On the surface of the spicules, look for the basophilic osteoblasts and the occasional osteoclast. Note the vascularity of the loosely arranged mesenchyme between the spicules. Find the developing periosteum, which will
eventually surround the entire bone. With time, the spicules will grow together to form trabeculae, and the trabeculae located just beneath the periosteum will grow together to form a region of compact bone. In the interior of the bone, the trabeculae will be retained in many areas to form spongy bone.

B. Endochondral Bone Formation

Slide 30 (HU Box): Chondroid Tissue, Human, l.s.
Slide 40 (HU Box): Developing Long Bone, l.s., or Slide 10: Finger

There are several versions of these slides. Try to find one that shows a good longitudinal section of a developing bone.

Endochondral bone formation occurs when bone tissue is laid down within and upon a pre-existing cartilage model of the bone. Eventually the cartilage is entirely removed and replaced by bone. The long bones of the body are examples of bones formed by endochondral ossification. Observing this slide with low magnification, you should see a light-stained epiphysis composed of hyaline cartilage. Earlier in development, this type of cartilage formed the entire model of the developing long bone. Will the epiphysis remain cartilaginous in the adult? [Answer: Not entirely. A secondary center of ossification will replace most of the epiphysis with bone, leaving hyaline cartilage only on the surface of the epiphysis that is exposed to the synovial cavity (articular hyaline cartilage).]

Identify the epiphyseal plate. Try to identify the following zones of the epiphyseal plate: the resting zone (cells are randomly distributed rather than lined up in columns), zone of proliferation (dividing cells form columns), zone of hypertrophy (cells enlarge), zone of calcification (cells have died and cartilage matrix has become calcified, i.e., more basophilic), and zone of ossification (spicules consist of eosinophilic bone matrix which has been laid down on bits of calcified cartilage to form mixed spicules). Metaphyseal blood vessels are prominent between spicules in the zone of ossification. You should also be able to identify osteoblasts, osteocytes, and osteoclasts.

Periosteum surrounds the bone externally, except at the articular surfaces.

C. Bone modeling

Bone modeling (A.K.A. growth remodeling, though your Ross textbook refers to this process as external remodeling) is the process by which bone increases in size and changes its shape. Bone modeling occurs mostly during growth and development (in subadults) though the process continues at a very slow rate in adults. A review of the slides you have previously studied can help you better understand this process.

Slide 5 (HU Box): Developing Membrane Bone, Human Fetus
Slide 48 (U. Michigan): Fetal Leg (See Virtual Microscope)
Slide 98 (HU Box): Bone C.S. and L.S.

Review Slide 5 (HÜ) and notice how many spicules of bone are lined with osteoblasts on one surface and osteoclasts on the opposing surface. Coordinated activity between these cells on neighboring bone surfaces allows the bone to change in size, shape and curvature through growth.

Understand that since slides 48 and 98 are long bone cross-sections, bone initially formed by endochondral ossification. However, growth in the width of the bone, the consolidation of the bone cortex, and the formation of the medullary cavity all involve modeling processes by which bone is added or removed at internal and external surfaces (a process technically similar to intramembranous ossification).
Notice how osteoblasts can be seen on surfaces where bone will form to fill in the large vascular spaces (forming primary osteons) located in regions that will become the bone cortex. Remnants of bone formed by endochondral ossification can be seen towards the center of the bone cross-section, as evidenced by spicules of bone with calcified cartilage cores. If the glycosaminoglycans of the cartilage matrix have been adequately preserved, the calcified cartilage stains more basophilic than the bone surrounding it, since bone contains fewer GAGs and more collagen.

D. Bone Remodeling

Slide 50 (University of Michigan)

Look for evidence of earlier bone remodeling such as interstitial lamellae or well-ordered Haversian systems, & for evidence of ongoing remodeling such as osteoclasts, resorption bays, and partially completed osteons.

E. Joints

In your study of cartilage, bone and bone formation, you have already looked at examples of two types of joints. When you studied fibrocartilage, you looked at an example of a cartilaginous joint called a symphysis, and when you studied bone formation, you looked at several examples of synovial joints. Use these slides again to study these joints in more detail.

Slide 45 (U Michigan Collection): Intervertebral Disk (See virtual microscope)

Symphyses are located along the mid-line of the body and can be found in the axial skeleton (e.g. intervertebral disks, manubrio-sternal joint). The pubic symphysis is the only example of a symphysis in the appendicular skeleton. The joint surfaces of a symphysis are covered by hyaline cartilage. Between the hyaline cartilage coverings is a layer of fibrocartilage.

In the intervertebral disk, notice the hyaline cartilage found immediately covering the bone surface (the end-plate) and the fibrocartilage forming the annulus fibrosus. The orientation of collagen fibers alternates between the fibrocartilage layers, giving the annulus fibrosis a lamellated appearance. Look deeper into the intervertebral disk to identify the inner nucleus pulposus.

Slide 10: Finger, or Slide 30(HU Box) and Slide 87 (HU Box): Knee, Rat, Methylene Blue

Synovial joints are characterized by a true joint cavity containing synovial fluid, which helps to lubricate the joint. They are lined by a synovial membrane and reinforced by a joint capsule composed of collagenous connective tissue, and often by several ligaments that are usually on the exterior of the capsule. Synovial joints are the most common type in the human body, and are significant because they permit extensive movement between the articulating bones. The surfaces of the bones enclosed within the joint capsule (the articular surfaces) are covered by articular cartilage. Articular cartilage is essentially hyaline cartilage, which lacks a perichondrium. Articular cartilage persists even after endochondral ossification has been completed.

In favorable sections where a joint is visible, articular cartilage covers the articulating surfaces of the bones. Locate the joint cavity and look for the synovial membrane. This is lined by a mixture of fibroblast-like cells and phagocytic cells rather than by epithelium. In the slide of the knee a portion of a meniscus (composed of fibrocartilage) is present. An epiphyseal plate can also be seen on some slides.
ELECTRON MICROSCOPY (RHODIN)

A. Endochondral Ossification

Review the process of endochondral ossification in light micrographs (Figs. 10-10 to 10-17, & 10-20 to 10-23). Compare the light and electron micrographs of the epiphyseal plate (Figs. 10-17 & 10-18). Identify the zone of proliferation, zone of hypertrophy, and zone of calcification.

B. Synovial Joints

Be able to identify the component parts of a synovial joint by LM (Figs. 9-27 to 9-30 & 10-22). Observe that articular cartilage is not covered by a periosteum (Figs. 9-27 & 9-28.) Study the nature of the synovial membrane that lines the inner surface of the capsule (Figs. 9-29 & 9-30). It contains two cell types: the B cells are fibroblasts that are thought to secrete the synovial fluid; the A cells are phagocytic cells that remove debris from the joint space. Fig. 9-30 contains part of a B cell recognizable by its RER. The other cell in this micrograph may be an A cell, but it is difficult to say for sure. You would like to see many more lysosomes in this cell in order to confirm its identification as an A cell.

LABORATORY 10 CHECKLIST
BONE FORMATION & JOINTS

<table>
<thead>
<tr>
<th>LIGHT MICROSCOPY</th>
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<tbody>
<tr>
<td>intramembranous bone formation</td>
<td>zone of hypertrophy</td>
</tr>
<tr>
<td>endochondral bone formation</td>
<td>zone of calcification</td>
</tr>
<tr>
<td>epiphysis</td>
<td>zone of ossification</td>
</tr>
<tr>
<td>diaphysis</td>
<td>bone spicules with a calcified cartilage core (mixed spicules)</td>
</tr>
<tr>
<td>epiphyseal plate</td>
<td>articular cartilage</td>
</tr>
<tr>
<td>resting zone</td>
<td>synovial membrane</td>
</tr>
<tr>
<td>zone of proliferation</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 10: BONE FORMATION & JOINTS

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. What evidence might you see in a section of demineralized bone that would allow you to conclude with certainty that the bone had formed by endochondral ossification?

2. How can you distinguish calcified cartilage from bone?

3. Briefly outline the morphological steps involved in intramembranous ossification.

4. What is a synchondrosi s? Give an example.

5. What is a gomphosis? Give an example.

6. Synovial membranes line all the surfaces of a synovial cavity that are not covered by articular cartilage. What is unusual about the types of cells that make up a synovial membrane?

7. What is a cutting cone?

8. A bone that is actively modeling usually includes sites along its inner and outer surfaces that are known as “reversals”. What is a reversal and how would you identify one morphologically?

9. Bone is one of the target organs for growth hormone. Which cells do you think growth hormone stimulates in order to produce an increase in the height of an individual?
LABORATORY 11
BLOOD & HEMATOPOIESIS

OBJECTIVES:

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

1. describe what a Romanovsky stain is

2. identify and describe mature blood cells at the LM and EM level:
   - erythrocytes
   - platelets (thrombocytes)
   - 5 types of leukocytes:
     - neutrophils
     - eosinophils
     - basophils
     - lymphocytes
     - monocytes

3. give the relative abundance of each cell type in normal peripheral blood

4. understand how the structure of each cell type reflects its function

5. identify the following features on a section of bone marrow:
   - vascular compartment
   - hematopoietic compartment
   - megakaryocyte
   - possible location of an erythroblastic islet

6. understand the distinction between red and yellow marrow

7. describe the vascular arrangement of bone marrow

8. identify and describe the following stages of erythrocyte differentiation:
   - basophilic erythroblast (basophilic normoblast)
   - polychromatophilic erythroblast (polychromatophilic normoblast)
   - orthochromatic erythroblast (orthochromatic normoblast)

9. identify and describe the following stages in granulocyte differentiation:
   - promyelocyte
   - myelocyte
   - metamyelocyte
   - band (stab) cell

10. identify the following cell types in electron micrographs of bone marrow:
    - megakaryocyte
    - orthochromatic erythroblast
    - reticulocyte
LABORATORY:

To study the structure of the marrow cavity use the sections of demineralized bone. These sections are usually so thick that the morphology of individual cells cannot be studied effectively, even under oil immersion. Therefore to study the morphology of individual cell types use the smears of peripheral blood and bone marrow, observing them first at low magnification and then with the oil immersion objective. Refer to the Connective Tissue lab to review the use of the oil immersion lens if necessary.

Please study the following slides:

I. Blood Smear
   Slide 33 (HU Box): Blood Smear, Wright-Giemsa Stain
   Slide 12: Blood Smear, Wright's Stain
   Start at low magnification and work your way gradually up to 40X before going to oil immersion. Find a good area of the slide where the red cells are a salmon pink color rather than green, gray or blue. Distinguish the anucleate red cells from the nucleated white cells (leukocytes). At 40x you can probably identify neutrophils and lymphocytes with relative ease. Monocytes, eosinophils & basophils are less common. You may want to scan the slide at 40x until you find a possible example of these cell types and then switch to oil to confirm the identification. If you want to switch back and forth between oil and 40x, remember to clean the oil off the slide before you go back down to 40x so that it won't get on the 40x lens. Clean the oil off the objectives and off the slide once you are done with it. Also identify the platelets. Platelets are not complete cells. Rather they are membrane-enclosed cytoplasmic fragments of cells called megakaryocytes.

   Do a differential count on 100 leukocytes to see if your slide has normal frequencies of each. Recognize that it is not possible to identify every leukocyte, since some have been damaged beyond recognition during the preparation of the smear. These are called smudge cells. Our slides are stained with Wright's stain or with both Wright's and Giemsa stain. Wright's and Giemsa are two examples of the family of stains known as Romanovsky stains that are commonly used on blood and marrow smears. Romanovsky stains are mixtures that include eosin, methylene blue and azure B. Methylene blue stains basophilic substances blue, eosin stains acidophilic structures pink, and azure dyes stain certain structures (such as azurophilic granules) a purplish-red color. In general, any structure that takes up azure dyes is described as azurophilic.

II. Bone Marrow
   Slide 7 (HU Box): Bone Marrow, Red, Section, H&E, or
   Slide 8A or 8B: Cancellous Bone
   Study one of these slides of sectioned bone marrow to recognize the overall organization of the marrow cavity. Distinguish the vascular compartment from the hematopoietic compartment. In which compartment will mature non-nucleated red cells be the predominant cell type? (Answer: In the vascular compartment) What cell type predominates in the other compartment? (Answer: In the hematopoietic compartment the majority of cells are granulocyte precursors). Each white, empty-looking circle in the hematopoietic compartment represents an extracted lipid droplet in the cytoplasm of a reticular (or adventitial) cell. These connective tissue cells and the reticular fibers they produce make up the stroma of the hematopoietic compartment. They also accumulate lipid when red marrow becomes yellow marrow. These stromal cells also regulate the
microenvironments that stimulate the development of different blood cell types and produce some of the growth factors that control the process of hematopoiesis. They differ from true adipocytes in many ways. For example, starvation will not cause lipolysis in lipid-laden reticular cells as it does in white adipocytes.

Marrow is predominantly red at birth, but becomes yellow marrow in most locations as the individual ages. Some red marrow can always be found in the normal adult, since there is a lifelong need to replenish aging blood cells. Based on the amount of lipid accumulated within the reticular connective tissue cells and the relative number of differentiating blood cells, decide whether your slides contain mainly red or yellow marrow. Finally recognize that it is also possible for yellow marrow to transform back into red marrow as a response to a sustained demand for additional blood cells.

In this slide you should also be able to identify megakaryocytes. What features of cellular morphology and location help to distinguish megakaryocytes from osteoclasts? (Answer: Osteoclasts have multiple nuclei and are located on internal or external surfaces of bone; megakaryocytes have one large lobed or folded nucleus and are more likely to be found near a venous sinusoid into which they can release platelets).

In the hematopoietic compartment, try to find a group of closely packed small cells with round, relatively heterochromatic nuclei. These are likely to be developing erythrocytes clustered around an inconspicuous macrophage to form an erythroblastic islet. These islets are normally only seen in sectioned marrow because they are fragile cell associations that usually break apart during preparation of a smear. The macrophage plays many roles in red cell development, including phagocytizing and destroying the nuclei that are extruded from the developing red cells.

Slide 13: Bone Marrow Smear, Red

DO NOT TRY TO IDENTIFY EVERY IMMATURE CELL YOU SEE. That would be a difficult task even for an experienced hematologist. Also do not try to find each cell in a developmental series in sequence (i.e., first the promyelocyte, then the myelocyte, then the metamyelocyte, etc.). This is much too difficult. Instead briefly evaluate each field and see if you can pick out any of the cell types you need to identify.

Try to find one or more good examples of the following cell types:

Red cell series:
- Basophilic erythroblasts
- Polychromatophilic erythroblasts
- Orthochromatric erythroblasts

Granulocyte series:
- Promyelocytes
- Myelocytes (neutrophilic myelocytes, eosinophilic myelocytes, and with a lot of luck basophilic myelocytes)
- Metamyelocytes (neutrophilic & eosinophilic)
- Neutrophilic band or stab cells

Platelet precursor:
- Megakaryocytes
Note: Since the nucleus of the eosinophil and the basophil do not become as highly segmented as that of the neutrophil, there is relatively little difference between a band and a mature cell in these lineages. Many authorities consider all such cells to be mature, and say that there is no basophilic or eosinophilic band stage.

To help you in identifying the cells listed above, you should evaluate features including the following:

- Cell size

- Whether there are any visible granules in the cytoplasm

- Size, abundance and staining affinity of any cytoplasmic granules

- Staining affinity (basophilia or eosinophilia) of cytoplasm itself (i.e., the cytosol surrounding the organelles)

- Shape of nucleus (round, lobulated, irregular, etc.)

- Chromatin pattern (euchromatin vs. heterochromatin; smudged vs. lacy)

- Number of nucleoli (zero to many)

- Nuclear:cytoplasmic ratio (for example there is very little cytoplasm relative to the nuclear volume in a small lymphocyte, but very abundant cytoplasm in a megakaryocyte)

The red cell series can be recognized by the fact that the cells are almost perfectly round, the nuclei are almost perfectly round at all stages, and the cytoplasm normally contains no granules except for a few very small purplish granules that represent lysosomes (even these are absent in the mature erythrocyte). The different cells in the developing red cell series are then distinguished by size (size decreases as you go from basophilic erythroblast to orthochromatic erythroblast) and by the color of the cytoplasm. Thus the basophilic erythroblast has the most intensely basophilic cytoplasm of any cell in the marrow, polychromatophilic erythroblasts have a distinctive grayish or lilac-colored cytoplasm, and orthochromatic erythroblasts have a pinkish orange cytoplasm close to the color of the mature erythrocyte. In late orthochromatic erythroblasts the nucleus becomes eccentric prior to being extruded.

It is difficult if not impossible on your slides to identify the stage of erythrocyte development known as the reticulocyte. With special stains such as cresyl blue you could identify them because you would see a bluish meshwork (a reticulum) in their cytoplasm. What is this meshwork composed of? (Answer: The stain causes the few remaining polysomes to clump together and precipitate, forming this meshwork. Because the polysomes degenerate within a day or two, older red cells don’t show this staining pattern.) Despite the similarity in their names, the reticulocyte is entirely unrelated to the reticular cell that forms the stroma of the hematopoietic compartment.

In the granulocytic line, the cells and nuclei are less likely to be perfectly round. The cell size decreases from promyelocyte to mature cell, and the nucleus becomes lobulated and more heterochromatic. Characteristics of each stage are as follows:

1. Promyelocyte:
   
   Multiple nucleoli, which usually look like a dark ring surrounding a lighter interior, rather than the uniformly dark nucleoli found in mature cells.
   
   Many small purplish azurophilic granules in the cytoplasm
   
   Basophilic cytosol
2. Myelocyte:
   In eosinophilic and basophilic lines, large specific granules appear in cytoplasm (myelocyte is therefore the first stage at which the 3 different types of granulocytes can be distinguished morphologically).
   In the neutrophilic line, specific granules are so small and poorly staining that they are usually not visible by LM; therefore cytoplasm often looks less granulated than in promyelocyte
   Nucleus relatively round or slightly flattened near cytocentrum
   Cytosol less basophilic

3. Metamyelocyte:
   All further changes involve change in nuclear shape. In metamyelocyte there is a deep U- or V-shaped indentation on the side facing the cytocentrum.

4. Band cell (stab cell):
   Nucleus looks like a curved dumbbell (two spherical terminal expansions separated by a curved cylinder of smaller diameter).

5. Mature granulocyte:
   The piece connecting the two lobes becomes thinner or irregular in diameter.
   In the case of neutrophils and some eosinophils additional lobes appear.

III. ELECTRON MICROSCOPY (RHODIN)

A. MATURE (PERIPHERAL) BLOOD
1. Observe the cytoplasmic granules in the neutrophil (Fig. 5-10; it is not necessary to distinguish between azurophilic granules and specific granules). Note that the neutrophil granules are much smaller than the cytoplasmic granules of eosinophils (Fig. 5-12) or basophils (Fig. 5-14).

2. Observe that the granules of human eosinophils contain an irregular crystalloid (Figs. 5-12 & 5-13). It is composed of a protein called major basic protein, and is probably the component that causes the intense eosinophilia of the granules. Such crystalloids are not seen in neutrophil or basophil granules. Major basic protein has anti-parasitic effects.

3. Monocytes have a well-developed Golgi complex containing several stacks of Golgi cisternae localized in the cytocentrum (Fig. 5-17). The Golgi is involved in the production of lysosomes, which these phagocytic cells use in heterophagy. The mature neutrophil is also phagocytic, but its Golgi is not well developed (Fig. 5-10). The neutrophil also has a more heterochromatic nucleus than a monocyte. The monocyte eventually differentiates into a macrophage in the connective tissues. Macrophages are even more phagocytic than monocytes and hence have even more Golgi complexes, lysosomes, and phagosomes, (Fig. 2-1).

4. Observe that a small lymphocyte has a heterochromatic nucleus, very little cytoplasm and very few organelles (Fig. 5-16). Small lymphocytes are cells that are currently not participating in immune responses. If they become activated in an immune response they enlarge, the nucleus becomes more euchromatic, and they develop a greater amount of cytoplasm containing a greater abundance of organelles. It is not possible by ordinary EM to distinguish between B and T lymphocytes, both of which can be of any size from small to large.
5. Compare the alpha granules and very dense granules of platelets (Figs. 5-19 to 5-21). Find the open canalicular system. The microtubules were not well preserved in these platelets (they disassemble if fixation is carried out on ice as it usually is). Normally they form a ring around the periphery of the platelet just beneath the plasma membrane. Their presence helps maintain the discoid shape of the platelet.

6. Be aware that mature red blood cells contain no nucleus and no cytoplasmic organelles (Fig. 5-7). What is the major component of the flocculent electron-dense material that fills the interior of RBCs? (Answer: Hemoglobin.)

B. HEMATOPOIESIS

1. Study the EM of an erythroblastic island (islet) shown in Fig. 6-22. Identify the macrophage and the erythroblasts associated with it. Within the cytoplasm of the macrophage notice several nuclei that were probably extruded from orthochromatic erythroblasts. They were phagocytized by the macrophage, which was in the process of destroying them at the time of fixation.

2. Fig. 6-27 illustrates the extrusion of the nucleus in the transition from orthochromatic erythroblast to reticulocyte. Note that the nucleus is surrounded by a thin rim of cytoplasm and by plasma membrane that will be pinched off from the cell along with the nucleus.

3. Fig. 5-8 shows a reticulocyte. It is not yet a mature red cell because it still contains some cytoplasmic organelles.

4. Fig. 6-31 illustrates the very large size of a mature megakaryocyte relative to the other cell types of the bone marrow. Note that although the megakaryocyte appears to have several nuclei these are actually all parts of a single highly folded nucleus. Again, trying to deduce nuclear morphology from a single thin section can be deceiving.

5. Observe the developing platelet demarcation channels and alpha granules within the cytoplasm of megakaryocyte precursors (Figs. 6-28 to 6-30). These demarcation channels fuse with one another and with the plasma membrane to wall off a portion of the cytoplasm that will become a platelet.

C. STRUCTURAL ORGANIZATION OF BONE MARROW (Fig. 6-5)

1. Identify the bone marrow sinusoid (part of the vascular compartment of the marrow cavity) and the hematopoietic compartment that contains developing blood cells. Maturing blood cells normally enter the vascular compartment by crossing the thin wall of such sinusoids.

2. Identify the reticular connective tissue cells (#4 in the figure). These cells are also called adventitial cells. In addition to the multiple functions noted above, these cells also form a discontinuous layer (labeled #3 in this micrograph) just external to the endothelium of the sinusoids, and are thus a component of the barrier which maturing cells must cross when they leave the hematopoietic cords and enter the circulating blood.
### LABORATORY 11 CHECKLIST
**BLOOD & HEMATOPOIESIS**

#### LIGHT MICROSCOPY

<table>
<thead>
<tr>
<th>Structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>erythrocyte</td>
<td>basophilic erythroblast</td>
</tr>
<tr>
<td>platelet</td>
<td>Polychromatophilic erythroblast</td>
</tr>
<tr>
<td>small lymphocyte</td>
<td>orthochromatic erythroblast</td>
</tr>
<tr>
<td>monocyte</td>
<td>Promyelocyte</td>
</tr>
<tr>
<td>neutrophil</td>
<td>neutrophilic myelocyte</td>
</tr>
<tr>
<td>eosinophil</td>
<td>eosinophilic myelocyte</td>
</tr>
<tr>
<td>basophil</td>
<td>basophilic myelocyte</td>
</tr>
<tr>
<td>vascular compartment of bone marrow</td>
<td>neutrophilic metamyelocyte</td>
</tr>
<tr>
<td>hematopoietic compartment of bone marrow</td>
<td>eosinophilic metamyelocyte</td>
</tr>
<tr>
<td>red vs. yellow marrow</td>
<td>neutrophilic band (stab) cell</td>
</tr>
<tr>
<td>megakaryocyte</td>
<td>azurophilic granules</td>
</tr>
<tr>
<td>osteoclast</td>
<td>specific granules of eosinophil</td>
</tr>
<tr>
<td>erythroblastic islet</td>
<td>specific granules of basophil</td>
</tr>
</tbody>
</table>

#### ELECTRON MICROGRAPHS

<table>
<thead>
<tr>
<th>Structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>erythrocyte</td>
<td>specific granules of basophil</td>
</tr>
<tr>
<td>platelet</td>
<td>erythroblastic islet</td>
</tr>
<tr>
<td>small lymphocyte</td>
<td>late orthochromatic erythroblast</td>
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<tr>
<td>monocyte</td>
<td>reticulocyte</td>
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<tr>
<td>neutrophil</td>
<td>megakaryocyte</td>
</tr>
<tr>
<td>eosinophil</td>
<td>platelet demarcation channels</td>
</tr>
<tr>
<td>basophil</td>
<td>bone marrow sinusoid</td>
</tr>
<tr>
<td>specific granules of eosinophil</td>
<td>hematopoietic compartment of bone marrow</td>
</tr>
</tbody>
</table>

**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 11: BLOOD & HEMATOPOIESIS

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. Which type of granulocyte is most abundant in peripheral blood? Which is least abundant?
2. Which type of agranulocyte is most abundant in peripheral blood? Which is least abundant?
3. Neutrophils and macrophages are both phagocytic, but only the macrophage has a well-developed Golgi. What role does the Golgi play in phagocytic cells such as macrophages, and why doesn’t a mature neutrophil need an extensive Golgi apparatus?
4. What is an azurophilic granule?
5. Which of the mature leukocytes is most likely to have a U- or horseshoe-shaped nucleus and a lacy (foamy) chromatin pattern?
6. Which of the formed elements of the blood (erythrocytes, platelets & the 5 types of leukocytes) normally carry out their functions within blood vessels?
7. With which of the formed elements of peripheral blood is each of the following most closely associated: spectrin, serotonin, myeloperoxidase, histamine, major basic protein?
8. How do basophils contribute to the swelling, redness, heat, and pain that are the hallmarks of an inflammatory reaction?
9. The enzyme carbonic anhydrase plays an important role in erythrocytes. It catalyzes the conversion of CO₂ to bicarbonate ion, the form in which most of the CO₂ is carried in the plasma. The reaction is:
   \[ \text{H}_2\text{O} + \text{CO}_2 \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{HCO}_3^- + \text{H}^+ \]
   What important role does this same enzyme play in osteoclasts?
10. What type of leukocyte would you expect to find in abundance in the connective tissue during the early stages of an inflammation caused by bacteria?
11. Name several roles that the macrophage plays in the differentiation of erythrocytes.
12. If you were harvesting marrow from an adult for use in a bone marrow transplant, from which of the following sites would you harvest it – sternum, iliac bone of the pelvis, midshaft of the humerus in the arm, or midshaft of the femur in the thigh? Why?
13. What color is the cytoplasm of a basophilic erythroblast, a polychromatophilic erythroblast and an orthochromatic erythroblast? What causes these changes in staining affinity?

14. What is the only stage of granulocyte maturation that contains both azurophilic and specific granules and is capable of division?

15. Which of the following are you most likely to see in a very immature undifferentiated cell such as the precursors of red and white blood cells that give rise to the morphologically identifiable stages: A heterochromatic nucleus, many prominent nucleoli, or a relatively large amount of cytoplasm (i.e. a low nuclear:cytoplasmic ratio)?

16. Why are the nuclei of developing red cells usually round rather than slightly flattened or indented on one side? Hint: What causes the flattening and indentation in cell types where it normally is seen?
LABORATORY 12
CARDIOVASCULAR SYSTEM
BLOOD VESSELS, LYMPHATICS & HEART

I. BLOOD VESSELS & LYMPHATICS

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

1. recognize the following types of vessels:
   - elastic artery
   - muscular artery
   - arteriole
   - capillary
   - postcapillary venule
   - muscular venule
   - vein
   - lymphatic capillary
   - larger lymphatic vessel

2. identify the following features of the wall in the appropriate types of vessels:
   - tunica intima
     - endothelium
     - internal elastic lamina (internal elastic membrane)
   - tunica media
     - smooth muscle cells
     - elastic fibers or sheets
     - external elastic lamina (external elastic membrane)
   - tunica adventitia
     - dense irregular connective tissue
     - vasa vasorum
     - nerves

3. compare and contrast the structure of arteries and veins. Note that the function of each vessel type is reflected in its morphology.

4. compare and contrast the ultrastructural features of:
   - continuous capillaries
   - fenestrated capillaries
   - sinusoids

   and give examples of locations where each is found

LABORATORY:

I. BLOOD VESSELS AND LYMPHATICS

The walls of capillaries are essentially composed only of endothelium. All other vessels are composed of several layers or tunics. The tunica intima (innermost layer) consists of endothelium and a thin connective tissue layer. The tunica media is the smooth muscle layer. It also includes different proportions of elastic and collagenous connective tissue depending on the vessel type. The tunica adventitia is a connective tissue layer that often blends into the connective tissue of surrounding structures.
Locate the various tunics in the vessels on the following slides. Recognize the composition of the layers in each vessel type and relate that composition to the physiologic function of the vessel.

In some of your slides you may observe an artifact common in sections of larger vessels, namely folds that form when the section is transferred to the glass slide and fails to spread out completely on the slide. The folds are dark straight lines, and are usually radially oriented. Multiple folds may form pleats at intervals around the vessel wall.

Please study the following slides in your set:

A. Muscular arteries
   Slide 47 (HU Box): Artery and Vein, or
   Slide 31A: Iliac Artery, H&E
   and
   Slide 31B: Iliac Artery, Aldehyde-Fuchsin Masson,
   Slide 32A (several versions): Renal Artery, Mallory Trichrome, or
   Slide 32B or 32C: Renal Artery, Elastic Stain

   Identify the three layers of the wall of an artery: tunica intima, tunica media & tunica adventitia. In an artery these three layers are fairly easy to distinguish from one another, that is, the wall of an artery is well-ordered. We will see later that the boundaries between layers in a vein are more ambiguous; i.e., the wall of a vein is less ordered. Note that smooth muscle is the only cell type in the tunica media and that it is circularly oriented. The tunica media is the most prominent layer in an artery, whereas the tunica adventitia is the most prominent layer in a vein. An artery usually travels with one or more companion veins (venae comitantes). The overall diameter of the veins is usually greater than that of the artery that they accompany, but the wall of the vein is thinner than that of the artery.

   Compare the appearance of muscular arteries stained with H&E, vs. a trichrome stain and an elastic stain. The internal elastic lamina (IEL) and the external elastic lamina (EEL) can be identified even in H&E preparations since they are relatively thick, but they stand out especially well in the sections stained for elastic fibers (slides 31B, 32A, 32B and 32C). Note that either of these laminae may be fragmented into more than one layer of elastic tissue. The EEL in particular is more likely to be present as multiple layers of elastic tissue that extend into the adventitia. Fragmentation tends to increase with increasing age. In the tunica media itself, the elastic tissue is fairly sparse, and is in the form of scattered elastic fibers. This is quite different from elastic arteries where the media contains abundant elastic tissue arranged as fenestrated elastic sheets (not individual fibers). These elastic sheets alternate in an orderly fashion with layers of smooth muscle cells. In the trichrome-stained specimen, the connective tissue and smooth muscle are stained different colors, making it very easy to distinguish the tunica adventitia (mainly connective tissue) from the tunica media (smooth muscle).

   When tissue is dropped into a fixative, the smooth muscle cells often contract. In muscular arteries this contraction can be strong enough to cause the endothelial cells to round up and the IEL to become wavy (“scalloped”). In life the IEL is not scalloped and the endothelial cells are typical squamous cells.

B. Elastic arteries
   Slide 45 (HU Box): Aorta, or
   Slide 29B: Aorta H&E
In an elastic artery, the media is composed of alternating layers of smooth muscle cells and sheets of elastic tissue (fenestrated elastic membranes). The elastic membranes are produced by the smooth muscle cells. Each muscle cell is anchored to the two elastic membranes between which it lies. The cells contract or relax to adjust the tension on the elastic membrane. Muscle contraction in an elastic artery thus has its greatest effect on the distensibility of the vessel wall; in muscular arteries and arterioles muscle contraction serves mainly to decrease lumen diameter. Locate the three tunics. Be sure you locate the endothelial surface of the vessel correctly. Elastic arteries include the aorta, the proximal part of the pulmonary arteries, and the proximal portions of the main aortic branches (brachiocephalic, common carotid, subclavian, common iliac and sometimes renal). Compare the appearance of an elastic artery in H&E, elastic stain and trichrome. Compare the structure of muscular arteries vs. elastic arteries.

C. Arterioles, Venules, Capillaries & Lymphatic Vessels

Venules come in two varieties: postcapillary venules and muscular venules. Postcapillary venules receive blood from capillaries, and consist of an endothelium and an incomplete layer of cells called pericytes located exterior to the endothelium. Pericytes are relatively undifferentiated and can give rise to cell types including smooth muscle. Muscular venules receive blood from postcapillary venules. Muscular venules have one or two complete layers of smooth muscle making up a true tunica media instead of a discontinuous layer of pericytes.

Capillaries have the smallest diameter of all blood vessels. They can be smaller than the diameter of a single erythrocyte, forcing the erythrocytes to deform in order to pass through the vessel. Capillaries have an endothelium, but no smooth muscle in their walls. Some may have scattered pericytes located exterior to the endothelium, as in postcapillary venules. The narrower diameter of the capillary distinguishes these vessels from the postcapillary venules.

Be sure to distinguish blood vessels from lymphatic vessels. The lymphatic capillaries have a wider, more irregular lumen than blood capillaries and have numerous valves, which blood capillaries lack. If cells are present in the lumen of the lymphatic capillary, they should be mainly lymphocytes rather than red blood cells (although sometimes rbcs artifactually spill from cut blood vessels into the lymphatics during tissue preparation). Larger lymphatic vessels have a wall that is even more disorganized than that of veins. It is very difficult to distinguish the tunica intima, tunica media and tunica adventitia from one another. There is greater variation in wall thickness around the circumference of a large lymphatic (some thick regions and some thin). Lymphatics and veins both have valves.
D. Thoracic Duct
Slide 35B: Thoracic Duct
This is the largest of all lymphatic vessels. Observe the extreme variations in wall thickness seen in a single cross-section. Note how difficult it is to distinguish the layers from one another. Find the circularly oriented smooth muscle. Most of this is in the media. You may also see bundles of longitudinally oriented smooth muscle that are typical of very large lymphatics. These may be in the adventitia and also in the intima, where they can form columns that give the lumen an irregular outline.

E. Large veins
Slide 35: Vena Cava (There are several versions).
Note that the tunica adventitia is the most prominent layer in the wall of a vein. The boundary between media and adventitia is especially prominent in the version of this slide that has been stained with Mallory trichrome. Observe that the adventitia has at least two zones: an inner one composed mostly of collagenous connective tissue, and an outer one that includes longitudinal bundles of smooth muscle. These longitudinal bundles are typical of very large veins, especially those below the level of the heart, which must return blood to the heart against gravity. Compare the organization of the wall of this vein with that of the thoracic duct in Slide 35B. It is somewhat easier to delineate the layers of the vein, and the thickness of the wall is more uniform in the vein.

Slide 35A: Pulmonary Vein
In the last part of the pulmonary vein, some of the muscle in the tunica media may not be smooth muscle. What type of muscle is it and how can you explain its presence there? (Answer: It could be cardiac muscle, since the pulmonary veins empty into the left atrium, and the transition from smooth to cardiac muscle does not always occur precisely at the junction of the vessel with the heart.) Check your slide to see what type of muscle is present in your specimen.

Slide 94B: Vagina
The veins in the wall of the vagina are particularly large in diameter, thin-walled and numerous. Their presence is one way of distinguishing vagina from other organs lined by minimally keratinized stratified squamous epithelium. During sexual arousal these veins become engorged with blood.

F. Sinusoids (Sinusoidal Capillaries)
Slide 7 (HU Box): Bone Marrow, Red, Section, or Slide 8A or 8B: Cancellous Bone
and Slide 10: Finger
Identify the sinusoids of the marrow cavity. Sinusoids (sinusoidal capillaries) are the most permeable type of capillary. They are characterized at the LM level by a lumen that is wider in diameter and more irregular than that of other capillary types. Sinusoids are found in relatively few organs such as bone marrow, liver, adrenal cortex and spleen. Their exact morphology varies significantly from organ to organ. At high magnification an individual sinusoid can be impossible to distinguish from a postcapillary venule by LM. However, if you observe a large enough part of the surrounding region
you should be able to distinguish between the two, since sinusoids (representing the capillary bed of the organ) are quite abundant, whereas postcapillary venules tend to be fewer in number and are more likely to be running with an arteriole.

Sinusoidal walls are extremely thin, and it is difficult to see them. Sometimes the sinusoids can be recognized by the fact they contain mostly RBCs, whereas the hematopoietic compartment that surrounds them contains mainly nucleated immature blood cells. For a clearer view of the sinusoidal wall, look at Slide 10. In that section from a fetal finger, hematopoiesis has just barely begun. As a result, the hematopoietic compartment is not crowded with developing cells. It consists mostly of mesenchyme, making it easy to locate the sinusoids.

**Slide 67 (HU Box): Liver, Stellate Reticuloendothel. Cells (Kupffer Cells), or Slide 68C, 68D or 68E: Liver**

Identify the liver sinusoids. They are unusual in that they have a lining that is composed of two types of cells: endothelial cells and Kupffer cells. Kupffer cells are macrophages that are scattered among the endothelial cells. It may be possible to identify Kupffer cells if they contain visible phagocytized debris. Kupffer cells also can span across the sinusoidal lumen and contribute to the wall on both sides of the vessel. This puts them in a better position to phagocytize debris passing through the vessel. The endothelial cells of liver sinusoids, in contrast, are squamous cells that do not stretch across the lumen.

II. **HEART**

**OBJECTIVES:**
At the end of this lab, you should be able to describe, and identify:
1. the endocardium, myocardium, and epicardium
2. the differences between the wall of an atrium vs. a ventricle
3. the following parts of the cardiac electrical conduction system:
   - SA node
   - AV node
   - Purkinje fiber
4. the structures associated with an atrioventricular (AV) valve:
   - chordae tendineae
   - papillary muscles
5. the basic structure of a semilunar valve
6. the cardiac skeleton

**LABORATORY:**

The heart consists of three main layers, the endocardium, myocardium and epicardium, which are analogous in position to the tunica intima, tunica media and tunica adventitia respectively. Like the tunica intima, the endocardium consists of an endothelium and underlying connective tissue. The myocardium contains cardiac muscle
rather than smooth. The cardiac muscle is attached to a cardiac skeleton composed of dense connective tissue. The epicardium, like the tunica adventitia is a connective tissue layer. Unlike the adventitia, epicardium contains large amounts of adipose tissue and is covered on its outer surface by a simple squamous epithelium (a mesothelium. The mesothelium, together with a thin underlying fibrous layer, forms the visceral layer of the serous pericardium. The coronary arteries and their large branches are located in the epicardium.

Blood flow through the chambers of the heart is controlled by valves. There are two types, the atrioventricular (AV) and semilunar valves, which differ in location and structure.

The rate of contraction is controlled and synchronized by an electrical conduction system in which the sinoatrial node (SA node) normally acts as pacemaker. The cells of the conduction system are modified cardiac muscle cells (not nerves). In addition, the heart receives extrinsic innervation from the autonomic nervous system. This innervation is not required for cardiac contraction, but it can modify the rate of contraction. The heart is nourished mainly by coronary arteries that arise from the aorta, and drained by coronary veins most of which drain into the right atrium.

Please study the following slides in your set:

Slide 36B (Several Versions): Right Heart Wall, or Slide 44 (HU Box): Cardiac Muscle

Begin by holding the slide up against a light background and orienting yourself before you study it under the microscope. Identify the thick wall of the ventricle, the thinner wall of the atrium, and if present, the AV valve. Then, observing through the microscope, locate the endocardium, myocardium, and epicardium in the two chambers. Note the difference in thickness of the corresponding layers in the atria vs. the ventricles.

Observe the smaller size of atrial cardiac myocytes compared to those of the ventricle. Look for cross-striations, intercalated disks, centrally located nuclei and branching of the muscle fibers. These features are best seen in the ventricular myocardium. Recall the differences between skeletal and cardiac muscle. The numerous capillaries in the myocardium reflect the rich vascularization of this layer.

Study the ventricle to see whether trabeculae carneae and chordae tendineae are present on your slide. You can distinguish between the two because they are composed of different tissues. What type of tissue makes up the core of the trabeculae carneae? (Answer: Cardiac muscle.) Of the chordae tendineae? (Answer: Dense regular connective tissue. Note that both trabeculae carneae and chordae tendineae are covered by endocardium.) If you are especially fortunate you may have a section that shows a chorda tendina anchoring to a papillary muscle. On the version of these slides that is stained with aldehyde-fuchsin and Masson trichrome, note that the base of the valve is anchored to a region of dense connective tissue that also separates the atrial and ventricular myocardium. This is part of the cardiac skeleton called an annulus fibrosus. It does not have a well-defined edge; rather it grades into surrounding looser connective tissue. Study the valve. Which layer(s) of the heart wall contribute(s) to the formation of a valve? (Answer: Only the endocardium contributes to the formation of a valve.) Observe the major branches of the coronary arteries in the epicardium. Look for evidence of pathological changes in these arteries such as thickened areas in the intima and fragmentation of the elastic laminae. What disease process may have caused these changes? (Answer: Atherosclerosis.) Observe the large amount of adipose tissue in the epicardium, especially in the AV sulcus.
Slide 36: Ventricle Wall
This slide shows the left ventricle (and in one version the left atrium as well). Compare the wall of the left ventricle with that of the right ventricle (slide 36B). Which is thicker? Why? [Answer: The wall of the left ventricle, especially the myocardial layer, is much thicker than the wall of the right ventricle. This reflects the fact that the left ventricle is part of a high-pressure system (the systemic circulation) while the right ventricle is part of a lower pressure system (the pulmonary circulation).]

Slide 48 (HU Box): Entire Heart, l.s.
Identify the atria and ventricles. The layers of the wall are not fully differentiated yet and are thus more difficult to distinguish from one another than in adult tissue. Concentrate instead on identifying the various chambers and distinguishing right side of the heart from the left. Many of these sections have passed through portions of the atria that contain pectinate muscles. These are seen as thick ridges that have been sectioned transversely. Notice how very thin the wall of the atrium is in the regions between pectinate muscles. Decide which is the left vs. the right ventricle. See if your section includes an elastic artery (the aorta) emerging from the left ventricle of the heart. If you are lucky your section may also show the pulmonary artery emerging from the right ventricle. Look for valve leaflets and decide if they belong to atrioventricular valves or semilunar valves. Find the interventricular septum.

Slide 39A: Heart, Purkinje Fibers
Purkinje fibers are modified cardiac muscle cells (not nerves) that contain few actin and myosin myofilaments and hence are poorly contractile. They are specialized for conduction of cardiac impulses rather than for contraction. They are larger in diameter than ordinary cardiac muscle cells and paler due to the paucity of myofilaments and also to the large amount of glycogen (usually lost during tissue preparation) that they contain. Purkinje fibers make up the distal portions of the AV bundles and eventually ramify among the ventricular myocytes and end on them, especially on the myocytes of papillary muscles. They can often be found grouped together in small bundles in the subendocardium. Look for the intercalated disks between Purkinje cells. Several histology textbooks claim that Purkinje fibers have no intercalated discs, but it would seem that they do indeed exist. Compare the diameter of the Purkinje cells to that of ventricular cardiac muscle cells. Which is wider? (Answer: The Purkinje fibers have a significantly wider average diameter than ventricular myocytes.) You should also be aware that there is a type of neuron in the brain called a Purkinje cell. It is a true neuron and is not related to the Purkinje fiber of the heart.

Slide 38: Sino-Atrial Node
In this section through the right atrium, the sinoatrial (SA) node is located in the thinner portion of the wall, between two branches of the nodal artery. The SA node is the portion of the cardiac conduction system that normally acts as the pacemaker. Like the Purkinje fibers, the cells of the SA node (and of the atrioventricular node) are modified cardiac muscle cells containing few myofilaments. However, whereas the Purkinje fibers are larger than ordinary cardiac myocytes, the cells of the SA and AV nodes are smaller in diameter than conventional myocytes. They are also separated from one another by more connective tissue than is usually seen between ordinary myocytes.

We have no slides of the AV node. By LM the cells would be very similar to those of the SA node. SA node and AV node would be distinguished mainly on the basis of
their location in the heart [SA node in the epicardium near the nodal artery and superior vena cava; AV node in the interatrial septum just superior to the right AV (tricuspid) valve].

III. ELECTRON MICROSCOPY (RHODIN)

A. Arteries

Observe in Fig. 16-26 that elastic laminae have openings (fenestrations) through which cell processes extend to establish contact and often gap junctions between cells (in this case between the endothelium & a smooth muscle cell in the media). Such junctions serve to metabolically couple the cells. Fig. 16-27 illustrates similar cell-to-cell contact and gap junction formation between smooth muscle cells in the media. In addition to metabolic coupling, junctions between muscle cells coordinate cellular contraction.

In Figs. 16-20 & 16-21 review the appearance of a collagen fiber (composed of many individual fibrils) and an elastic fiber (composed of many thin microfibrils embedded in an amorphous matrix of elastin).

B. Arterioles (Fig. 16-30)

This example has an incomplete internal elastic lamina. In most arterioles with only one layer of smooth muscle, both internal and external elastic laminae would be absent. This vessel is clearly an arteriole since it doesn't have enough layers of smooth muscle to be a muscular artery.

C. Capillaries

Fig. 16-35 shows an EM of a capillary bed. The arrows indicate the direction of blood flow. Identify endothelial cells, capillaries with their narrow lumens, and postcapillary venules with wider lumens.

By EM three types of capillaries can be identified: continuous capillaries, fenestrated capillaries and sinusoids (sinusoidal capillaries). Study the following examples and be able to distinguish between them.

Continuous capillaries (Figs. 16-36 &16-38 A & B) are distinguished by an endothelial cytoplasm that lacks fenestrations and has no large gaps between cells or within the cytoplasm of individual cells. As a result the cells form a continuous lining around the capillary lumen. Note the presence of micropinocytotic vesicles, which are important in transporting materials across continuous capillaries. Continuous capillaries are found in muscle and alveolar capillaries of the lung.

Fenestrated capillaries (Figs. 16-37 & 16-38 C) have many small (80-100 nm diameter) pores or fenestrations in their cytoplasm. These are usually covered not by cytoplasmic membrane but by a thinner layer called a diaphragm. The diaphragm is composed of proteins that form channels that regulate the permeability of the fenestrations. Fenestrated capillaries are common in organs such as endocrine glands where increased vessel permeability is important. Note that in the glomeruli of the kidney the fenestrated capillaries are unusual in that they lack diaphragms (Fig. 16-38 D). Part of one endothelial cell is shown in the upper half of this micrograph. It is separated by a thick basement membrane.
from cytoplasmic processes belonging to the epithelial cells of Bowman's capsule (podocytes) (#7 in the micrograph).

Sinusoids (Fig. 16-38 E) are characterized by large gaps (#12) between neighboring cells (#2), by groups of fenestrations in some parts of their cytoplasm, and by even larger openings through the cytoplasm of individual endothelial cells, like the holes in a piece of Swiss cheese. The basal lamina is often discontinuous, and is not seen in this micrograph. Sinusoids are found in only a few organs including liver and bone marrow.

D. Postcapillary Venules (Figs. 16-42 & 16-43)
Note the wider diameter of the postcapillary venule vs. the capillaries. Find the pericytes that can be found associated with either postcapillary venules or capillaries. They do not form a continuous layer as smooth muscle cells do.

E. Muscular Venules
Muscular venules (Fig. 16-46) have a continuous layer of smooth muscle surrounding the endothelial cells. They resemble arterioles, but usually have a wider lumen. Compare the thickness of the endothelial cell nucleus with the width of the lumen in Figs. 16-30 (arteriole) and 16-46 (muscular venule).

F. Veins
Compare a companion artery-vein pair (Fig. 16-24). The wall of the arterial vessel is thicker than that of the companion vein, and the layers are much easier to identify in than in the vein. Which of these vessels would have the wider lumen? (Answer: In a companion pair, the vein would usually have a wider lumen than the artery.)

G. Lymphatic Vessels
Compare the structure of lymphatic capillaries with that of blood capillaries (Fig. 17-2). Which type of vessel usually has a wider lumen? Which usually has more regularly shaped (i.e., circular) lumen in cross sections? (Answer: The lumen of the lymphatic capillary is usually wider and the wall is thinner than in a blood capillary. The blood capillary would tend to have the more regular circular shape in cross-section.)
Observe the structure of the lymphatic valve shown in Fig. 17-5. It is lined on both sides by endothelium with a small amount of connective tissue between them. The valves of veins have a similar structure although the connective tissue core is often thicker.

H. Heart:
The basic ultrastructure of cardiac muscle cells has been studied in a previous lab. Review the structure of an intercalated disc, including fasciae adherentes, desmosomes, and gap junctions (Figs. 11-27 & 11-29).
Compare the size of Purkinje fibers to ordinary ventricular cardiac muscle cells (Fig. 11-34). Notice how few myofibrils (#6) there are in the Purkinje fibers. In life, Purkinje fibers tend to contains large amounts of glycogen. It is mostly extracted during fixation, contributing to the pale staining of the cytoplasm.
Study the AV valve in Fig. 16-10, noting that it is formed by a fold of endocardium and therefore has endothelium lining both surfaces and a core of connective tissue. In Figs. 16-11 & 16-12 observe a chorda tendineae in longitudinal and cross section. Their surface is covered by endothelial cells, while their core is composed of connective tissue. In a trabecula carnea, cardiac muscle would form the core.
**LABORATORY 12 CHECKLIST**  
**CARDIOVASCULAR SYSTEM**

### LIGHT MICROSCOPY

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>tunica intima</td>
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### ELECTRON MICROGRAPHS

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<td>lymphatic capillary</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 12: CARDIOVASCULAR 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. In connective tissues, fibroblasts produce most of the components of the extracellular matrix, but in the tunica media of blood vessels smooth muscle cells carry out this function. This may be a reflection of the fact that both cell types develop embryologically from ______________.

2. In the tunica media of blood vessels, not every smooth muscle cell is directly innervated by a nerve. In the absence of direct innervation how can these muscle cells contract?

3. What type of vessel should you associate with each of the following functions in the vascular system: cellular exchange between blood and extravascular tissue; recovery of excess fluid from the extravascular space; regulation of blood pressure?

4. In elastic arteries, contraction of smooth muscle doesn’t have a major effect on decreasing the diameter of the vessel as it does in arterioles. What does smooth muscle contraction achieve in the walls of elastic arteries?

5. Histamine from activated basophils and mast cells has important effects on endothelial cells and vascular smooth muscle. What are these effects and in what way are they beneficial? How can they become harmful?

6. What component of the wall of a blood vessel is especially effective in activating platelets? How does this relate to platelet function?

7. What are the three parts of the cardiac skeleton? What type of tissue are they composed of? What functions do they serve?

8. What is the significance of the fact that there is no direct continuity of ordinary cardiac muscle between the atria and the ventricles of the heart?

9. Why are cells of the cardiac conduction system (e.g., Purkinje cells, SA nodal cells) paler than ordinary cardiac myocytes? How does this relate to the function of these cells?
LABORATORY 13
LYMPHOID ORGANS
THYMUS, LYMPH NODES & SPLEEN

Lymphoid tissue can be defined as tissue in which the major parenchymal cell type is the lymphocyte. Lymphoid tissue is involved in immune responses. It can be found in the lymphoid organs and also as a normal part of nonlymphoid organs, especially in the GI tract, the urogenital (UG) tract and the respiratory system. The lymphoid organs include the thymus, lymph nodes, spleen and tonsils. When present in nonlymphoid organs, lymphoid tissue can be found as diffuse lymphocytic infiltration, as individual nodules, or as aggregated nodules such as those of the appendix or Peyer’s patches. Most lymphoid tissues contain mixtures of both major types of lymphocytes: B cells and T cells. Note that these cannot be distinguished from one another by routine light or electron microscopy. Plasma cells, which are the antibody-producing cells derived from B cells during an immune response, are also found in lymphoid tissues, as are macrophages, which play important accessory roles in immune reactions.

In this laboratory we will study the thymus, lymph nodes and spleen. The lymphoid components of other organs systems such as the GI tract will be studied later when we consider each of those systems.

I. THYMUS:

OBJECTIVES:
At the end of this lab, you should be able to:
1. distinguish cortex from medulla, and understand why the medulla stains paler than the cortex with H & E
2. list the structural elements of the blood-thymus barrier and discuss its functional significance
3. describe the pathway followed by the maturing thymocytes as they pass through the thymus
4. describe the type of stroma that supports the thymus
5. identify Hassall’s corpuscles; know where they are located, and what cell type forms them
6. describe the histological differences between a newborn thymus and an involuted thymus

LABORATORY:

Please study the following slides in your set:
- Slide 52 (HU Box): Thymus, c.s., or
- Slide 23: Thymus Gland, Human Infant

The thymus has a thin connective tissue capsule. It is divided into two lobes, which are incompletely subdivided into lobules by connective tissue septa that extend inward from the capsule. Each lobule has a darkly staining cortex (the outermost zone), and a paler staining medulla (the central zone). The medulla is continuous from one lobule to the next, thus forming the central core of the organ.
Immature T cells within the thymus are called thymocytes. They are derived from prothymocytes from the bone marrow that enter the thymus by crossing the walls of blood vessels near the corticomedullary boundary. As they mature they move first to the outer cortex (just beneath the capsule) dividing relatively rapidly as they go, then return to the deep cortex, enter the medulla, and finally leave the thymus via postcapillary venules &/or lymphatics located near the cortico-medullary boundary.

The thymus contains several other major cell types including: macrophages and thymic epithelial cells (TECs), also called epithelial reticular cells (ERCs), or epithelial stromal cells. Macrophages are large pale cells that can sometimes be identified if phagocytized cell debris is visible in their cytoplasm. Why are many macrophages needed in the thymus? (Answer: Most thymocytes die in the thymus during the process of maturation. Macrophages phagocytize and destroy the apoptotic bodies that are the remains of the dead thymocytes.) TECs are also large and euchromatic but unlike macrophages they are essentially non-phagocytic. The thymus is unique among the lymphoid organs in having a stroma that is composed of epithelial cells rather than reticular cells. In the medulla, concentric layers of TECs form Hassall's corpuscles (thymic corpuscles), which act indirectly in the production of regulatory T cells.

The thymus is an example of a primary lymphoid organ (i.e., an organ in which lymphocytes differentiate but do not carry out the functions of mature lymphocytes). One morphological reflection of this fact is that the thymus does not normally contain lymphoid nodules. As we will see shortly, lymphoid nodules represent locations where mature B lymphocytes are preferentially located and where activated B lymphocytes divide and mature during an antibody-producing immune response.

Slide 24: Thymus Gland, Human Adult
Compare the involuting thymus on this slide with that of the previous slide. Note that involution involves the replacement of much of the lymphoid tissue of the thymus by adipose tissue. The cortex is usually affected before the medulla, so that in some places you may see lobules consisting of medulla with little or no associated cortex.

II. LYMPH NODES:
OBJECTIVES
At the end of this lab, you should be able to:
1. identify outer cortex, deep cortex (paracortex), medulla and hilus
2. explain the difference between a node and a nodule (follicle)
3. distinguish between a primary and a secondary nodule; understand the significance of a germinal center
4. distinguish between medullary cords and medullary sinuses
5. identify the regions within a node where the following cell types preferentially localize: B cells, T cells, plasma cells
6. describe the system of lymphatic sinuses within a node
7. describe the type of stroma that supports lymph nodes
8. describe how biological & mechanical filtration both contribute to the removal of antigens from the lymph within a lymph node
9. describe how the morphology of an inactive node changes during an immune response
Lymph nodes are small lymphoid organs that are connected in series by lymphatic vessels; their major role is to remove antigens from the lymph and initiate immune responses to them. The human body contains hundreds of lymph nodes arranged in groups that are usually found near major veins.

A connective tissue capsule surrounds each lymph node, and finger-like extensions called trabeculae extend inwards from it. Reticular fibers are anchored to the trabeculae and inner surface of the capsule. They are produced by reticular connective tissue cells which remain adherent to them. The reticular cells and fibers together form the stroma of the lymph node.

A lymph node is divided into an outer region called the cortex, and a central region called the medulla. The cortex is characterized by many closely packed lymphocytes and relatively few lymphatic sinuses, and therefore appears darker-staining and more solid than the medulla. The medulla includes medullary sinuses and medullary cords. Medullary sinuses are wide, light-staining lymphatic sinuses. They are numerous and may comprise up to half the volume of the medulla. Medullary cords are the areas of tightly packed cells between medullary sinuses. The cords contain a mixed cell population including B cells, T cells, macrophages, and most of the plasma cells of the node.

Lymph enters a node via numerous small afferent lymphatic vessels, which empty, into the subcapsular sinus located between the capsule and the cortex. From there much of the lymph flows through a network of lymphatic sinuses that are arranged as follows: from subcapsular sinus to the trabecular sinuses which surround the trabeculae in the cortex, to medullary sinuses and finally, near the hilus, to efferent lymphatic vessels. The hilus is the region where major blood vessels enter and leave the node and where the efferent lymphatics leave. It can be identified by the presence of these major vessels and by the fact that at the hilus there is no cortex, and the medulla is thus in direct contact with the capsule. Note that not every section goes through the hilus, so you may not be able to find it on every slide.

The cortex can be further subdivided into an outer cortex and a paracortex (also called deep cortex). The outer cortex consists of lymphoid nodules and the regions between nodules, which are called the internodular cortex. The paracortex is an area that lacks nodules. The paracortex is sometimes considered to be a separate third layer of the node (cortex, paracortex & medulla) rather than a subdivision of the cortex. B lymphocytes are preferentially localized within nodules, while T lymphocytes are preferentially localized in the internodular cortex and the paracortex.

There are two morphological types of lymphoid nodules: primary nodules and secondary nodules. Primary nodules consist of tightly packed small lymphocytes and are uniformly dark-staining throughout. Secondary nodules have a paler central region known as a germinal center surrounded by a darker staining region called the cap or mantle. A germinal center represents a location where activated B lymphocytes are proliferating as part of an antibody-producing immune response. Therefore a lymph node in which such an immune response is occurring will have many secondary nodules. A lymph node in which no antibody production is occurring will have primary nodules. Try to find a mitotic figure within a germinal center.
Germinal centers are also sites of extensive lymphocytic cell death. Numerous macrophages are present within them to dispose of the dying cells. The macrophages are large pale cells, often with visible phagocytized cellular debris. Macrophages also act as antigen presenting cells (APCs) to help process antigen and present the processed fragments to lymphocytes to initiate an immune response.

The paracortex contains unusual postcapillary venules that have a “high” endothelium. Their endothelial cells, instead of being squamous, are cuboidal to low columnar. Such vessels are called high endothelium venules (HEVs). They are difficult to identify by light microscopy, but are functionally important since most of the lymphocytes within a node got there by migrating between high endothelial cells to leave the lumen of an HEV and enter the paracortex. The Endothelial cells of an HEV are pale-staining and hard to see in the crowded conditions in the paracortex. With luck, they will be easier to find on the next slide.

Slide 71B: Pancreas, Newborn

In this slide study the developing lymph nodes surrounding the pancreas, not the pancreas itself. The nodes are at a very early stage of formation and not much their typical structure is recognizable yet (usually only a hint of the capsule and subcapsular sinus). However, the high endothelium venules (HEVs) are well-developed at this time since so many lymphocytes are migrating into the node during its formation. If the nodes on your section contain any good HEVs, it will be immediately obvious.

Slide 26B: Lymph Node, Reticular Fiber

This silver-stained preparation reveals the distribution of the reticular fibers within a node. These fibers remain closely associated with the reticular cells that produced them, and together they form the stroma that supports the node. Macrophages often adhere to this stroma, thus becoming what is referred to as “fixed” rather than free macrophages. The fibers are easily visible in silver-stained preparations, but the cells are difficult to find. Notice that reticular fibers are present within the cortex (although they are relatively rare in germinal centers), the medulla, the trabeculae, and also within the lumen of the lymphatic sinuses. The stromal elements form an efficient mechanical filter that physically traps particulate matter. The fixed macrophages act as a biological filter that phagocytizes foreign matter. Both help remove antigens from the lymph as it passes through the node. Notice that, unlike the sinuses located within the node, afferent and efferent lymphatic vessels do not have these stromal elements strung across their lumens. Afferent and efferent sinuses also contain valves, while the sinuses within the node do not.

III. SPLEEN:

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

1. distinguish between the red pulp, white pulp, and marginal zone
2. identify the component parts of the white pulp (PALS, lymphoid nodules) and central artery
3. identify the component parts of the red pulp: splenic sinuses, and splenic cords (Billroth’s cords)
4. list the part(s) of the spleen to which B cells preferentially migrate; list the parts to which T cells preferentially migrate
5. describe what the term “open” circulation means
6. understand how the structure of splenic sinuses is unusual, and how this contributes to the destruction of old red cells in the spleen
7. compare the structure of the spleen to that of the thymus & lymph nodes

LABORATORY
Slide 50 (HU Box): Spleen, or Slide 28B or 28C: Spleen
The spleen is a secondary lymphoid organ that is specialized to deal with antigens carried in the blood. At low magnification, two zones can be identified by their different staining affinities:

Red pulp (appears reddish due to large numbers of erythrocytes)
White pulp (appears bluish due to large numbers of lymphocytes)

Identify the following two components of the red pulp:
1. Venous sinuses (splenic sinuses). These are sometimes difficult to see by LM since the walls are very thin. Since they are vessels containing peripheral blood, you can try to locate them by looking for areas where there are large numbers of mature erythrocytes. If the blood has been washed out by fixing the tissue via perfusion, the sinuses will appear as empty, open spaces.
2. Splenic cords. These are areas of closely packed splenic parenchymal cells between adjacent venous sinuses. They will contain some blood, but also many additional lymphocytes, macrophages, reticular stromal cells, and plasma cells.

Next identify the three components of the white pulp:
1. Periarteriolar lymphatic sheath (PALS), which is composed mainly of closely, packed T lymphocytes. A cylindrical sheath of PALS surrounds every central artery.
2. Central arteries, which are branches of trabecular arteries.
3. Lymphoid nodules (follicles). As in other lymphoid tissues and organs, nodules in the spleen represent areas where B lymphocytes preferentially localize. Scattered primary and secondary nodules are embedded at irregular intervals along the PALS. The nodules may displace the central artery so that it becomes pushed to the edge of the PALS rather than centrally located.

Note that since the white pulp is always organized around a central artery, and since there are many central arteries scattered throughout the spleen, the white pulp exists as many separate islands of tissue each surrounded by red pulp, rather than as one continuous region.
Locate the capsule of the spleen. It is a fibroelastic connective tissue containing occasional smooth muscle cells. A layer of mesothelial cells covers the surface of the capsule, since the spleen is an intraperitoneal organ.

Slide 28A: Spleen, Reticular Fiber
The stroma of the spleen, like that of the lymph nodes, consists of reticular cells and the reticular fibers that they produce. This specimen is silver-stained to reveal the reticular fibers. These are numerous in the trabeculae, around blood vessels and throughout the red and white pulp (except for the germinal centers where they are rare). They are not present in the lumen of the splenic sinuses.

Slide 51 (HU Box): Reticular Fibers, Silver Stain
Some versions of this slide were prepared from spleen and others from lymph node. Using your knowledge of the general architecture of lymph node vs. spleen, determine which organ you have on your slide.

IV. ELECTRON MICROSCOPY (RHODIN)

A. THYMUS:
1. Thymic epithelial cells (TECs) (Figs. 19-7 & 19-8). Two features that distinguish these cells from macrophages are: the desmosomes that join neighboring TECs together and the bundles of tonofilaments in their cytoplasm. These features are common in epithelia but are not found in macrophages or other connective tissue cells. Note that some types of TECs have long thin cytoplasmic processes that surround the thymocytes. Others, such as those that line the inner surface of the capsule, or separate the cortex from medulla, are more squamous in shape.
2. Thymic macrophages (Figs. 19-4 & 19-7): Look for phagocytized debris within their cytoplasm. They are responsible for removing the many thymocytes that die within the thymus.
3. Dying thymocytes. Thymocytes die through a process called apoptosis, which is characterized morphologically by margination of heterochromatin, the fragmentation of the nucleus, and blebbing to form apoptotic bodies that are phagocytized by macrophages.

B. LYMPH NODE:
1. The lymph sinuses within the node (Figs. 17-14, 17-15 &17-18): Note that the endothelial lining of the sinuses is not continuous (Fig. 17-15), making it very easy for lymph to leave these vessels and percolate between the lymphocytes of the cortex and medulla. Note also that the reticular connective tissue stroma is present within the lumen of all the intranodal sinuses.
2. Germinal centers (Figs. 17-14 & 17-16): Compare the size of the lymphocytes in the germinal center with those of the surrounding cap (mantle or corona). Notice the numerous macrophages of the germinal center.
3. **Medullary cords**: Medullary cords are nothing more than the regions between lymphatic sinuses (Fig. 17-18). They are filled with closely packed lymphocytes, macrophages, and plasma cells (Figs. 17-30 & 17-31). Plasma cells are most numerous when a humoral immune response is in progress.

4. Observe the structure of high endothelium venules (HEVs) (Fig. 17-33). They are lined by a simple cuboidal to columnar endothelium. It is not uncommon to see several lymphocytes in the process of crossing the wall of such vessels. In which direction are they going? *(Answer: They are moving from the blood into the paracortex of the node, where immune responses are often initiated.)* Whereas all leukocytes can cross the wall of ordinary postcapillary venules, passage across the walls of these HEVs seems to be restricted mainly to T and B lymphocytes.

C. **Spleen**:
   1. Compare the components of the red pulp, white pulp and the marginal zone of the spleen (Fig. 18-6). The white pulp contains a central artery (#1) and its branches (#2); the major cell type is the lymphocyte. The red pulp has wide thin-walled splenic sinuses (#5), and splenic cords (#6) that contain many erythrocytes. The marginal zone is the transitional region between red and white pulp. It contains small splenic sinuses (#3) and a mixture of lymphocytes and blood cells.

   2. Examine the ultrastructural evidence for an open circulation in the spleen (Figs. 18-9 & 18-13), i.e., identify the endothelial cells lining the blood vessels and see if you can find the point where the vessel appears to end and dump the peripheral blood into a splenic cord. In Fig. 18-13 identify two splenic sinuses that the blood cells appear to be re-entering.

   3. Examine the ultrastructural details of the splenic sinuses (Figs. 18-13, & 18-17 to 18-20). Identify the structural components of the barrier that must be crossed by red cells as they move from the splenic cords into splenic sinuses: adventitial or reticular cells, the hoops of the discontinuous basement membrane, (#5 in Figs. 18-19 & 18-20), and the elongated cigar-shaped endothelial cells. Notice the many macrophages in the splenic cords that can phagocytize fragmented erythrocytes.
# LABORATORY 13 CHECKLIST

## LYMPHOID ORGANS

### LIGHT MICROSCOPY

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<tr>
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### SPLEEN:

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<tr>
<td>PALS</td>
<td></td>
</tr>
<tr>
<td>central artery</td>
<td></td>
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</tbody>
</table>

### ELECTRON MICROGRAPHS

<table>
<thead>
<tr>
<th>THYMUS:</th>
<th>SPLEEN:</th>
</tr>
</thead>
<tbody>
<tr>
<td>thymic epithelial cell (TEC)</td>
<td>red pulp</td>
</tr>
<tr>
<td>apoptotic thymocyte</td>
<td>splenic cord</td>
</tr>
<tr>
<td>LYMPH NODE:</td>
<td>splenic sinus</td>
</tr>
<tr>
<td>subcapsular sinus</td>
<td>marginal zone</td>
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<tr>
<td>medullary sinus</td>
<td>white pulp</td>
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<tr>
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<td>central artery</td>
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<tr>
<td>germinal center</td>
<td>germinal center</td>
</tr>
<tr>
<td>high endothelium venule (HEV)</td>
<td>PALS</td>
</tr>
</tbody>
</table>

**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 13: LYMPHOID ORGANS

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. What are primary lymphoid organs?
2. What are secondary lymphoid organs?
3. When a lymphocyte has been activated in an immune response, it enlarges to become a large lymphocyte, and then divides repeatedly. This is referred to as blast transformation and the large dividing cells are called immunoblasts. Why is it important for the activated lymphocytes to undergo multiple divisions?
4. Why is it advantageous for lymphocytes to recirculate through the body rather than remaining in one location permanently?
5. What good does it do for an antibody to bind to an antigen? How does this result in destruction of the antigen?
7. Do thymic epithelial cells (TECs) have any other functions in the thymus beyond providing physical support?
8. Is it correct to say that once the thymus involutes it no longer produces T cells?
9. How do most antigens enter lymph nodes? How do lymphocytes enter lymph nodes?
10. Lymph is not just a nuisance that has to be dealt with in order to avoid edema. In what way does the flow of lymph make the immune system more efficient in dealing with antigens?
11. If the lymph nodes fail to completely eliminate an antigen from the lymph, where does the antigen go next? What organ serves a “back-up” function in that it can mount an immune response against the remaining antigen?
12. What is meant by an open circulation vs. a closed circulation?
13. Describe the alternative theories regarding the path of blood flow through the spleen.
14. How does the structure of splenic sinuses differ from that of other sinusoids? How does their structure reflect their function in the destruction of senescent red cells?
15. Why are central arteries often not centrally located in the white pulp of the spleen?
16. What lymphoid organ contains Hassall’s corpuscles? Afferent lymphatics? High endothelium venules (HEVs)? A marginal zone?
LABORATORY 14
RESPIRATORY SYSTEM

OBJECTIVES:

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

1. describe the structure of the conducting airways (nasal cavity, larynx, trachea, bronchi, and conducting bronchioles), and relate their structure to their functions

2. describe the structure of the respiratory (exchange) airways (respiratory bronchioles, alveolar ducts, alveolar sacs and alveoli), and relate their structure to their functions

3. explain the concept of a bronchopulmonary segment

4. describe where branches of pulmonary vein, pulmonary artery and bronchial artery are usually located relative to the airways

5. describe the structure of an alveolus and an interalveolar septum; know the components of the air-blood barrier

6. identify the following epithelial cell types by LM and TEM:
   the olfactory epithelium, including:
   olfactory receptor cells (sensory cells)
   supporting cells
   basal cells
   the respiratory epithelium, including:
   ciliated cells
   goblet cells
   Clara cells (in bronchioles)
   the alveolar epithelium, including:
   alveolar type I cells (squamous alveolar cells)
   alveolar type II cells (great alveolar cells)
   alveolar macrophages (dust cells)

7. describe the source and function of surfactant

8. distinguish between the false and true vocal folds of the larynx

LABORATORY:

In this laboratory, you should become familiar with the histology of the conducting vs. respiratory (exchange) portions of the respiratory system. Relate morphology to function as you examine regions concerned with olfaction, humidification, speech, maintenance of a patent airway, respiratory exchange, and removal of particulate matter.
Please study the following slides in your set:

**Slide 6 (HU Box): Olfactory Epithelium**

This slide contains both respiratory epithelium and olfactory epithelium. Both are pseudostratified columnar, but they can be distinguished because olfactory epithelium lacks goblet cells and is thicker (taller) than the respiratory epithelium adjacent to it (Ross, Fig. 19.3b, p. 667 vs. Fig. 19.9, p. 675). Olfactory epithelium is found in a relatively small area near the roof of the nasal cavities (Ross, Fig. 19.2, p. 666). Realize that the olfactory epithelium contains three cell types: sustentacular (supporting) cells, basal cells and olfactory receptor cells (Ross, Fig. 19.3a). The olfactory receptor cells are really bipolar neurons. Their nonmotile cilia carry the receptors for odors. Although it is usually not possible to distinguish between these cell types with certainty by LM, their nuclei do have characteristic locations within the epithelium. Thus the nuclei located closest to the epithelial surface usually belong to supporting cells, while basal cell nuclei are closest to the basement membrane, and the nuclei in an intermediate position belong mainly to olfactory receptor cells (or to immature sustentacular cells that have not yet reached their full height).

Olfactory epithelium is also characterized by serous glands called Bowman’s glands (located just beneath the epithelium), and by the presence of relatively large nerve bundles representing the central processes of the olfactory receptor cells. These make up the olfactory nerve (cranial nerve I). They will pierce the ethmoid bone and enter the olfactory bulb of the brain. Notice that glands may also be associated with the respiratory epithelium, but they are usually mixed muco-serous glands.

**Slide 18 (HU Box): Epiglottis, l.s.**

The core of the epiglottis is elastic cartilage. Recall from the cartilage lab that some of these slides show fatty degeneration of the epiglottic cartilage. The epithelium covering the epiglottis is sometimes different on its two surfaces. The upper (anterior or lingual) surface is lined by a minimally keratinized stratified squamous epithelium (a wear and tear epithelium) that is continuous with the same type of epithelium in the oropharynx. Early in life its posterior (inferior or laryngopharyngeal) surface may be at least partly covered by respiratory epithelium that is continuous with lining of the vestibule of the larynx. In most of our slides both surfaces are covered by a stratified squamous epithelium. This is typical of smokers, older individuals, or individuals who subject the larynx to unusual wear and tear. It is an example of metaplasia (the change of one type of normal epithelium into another normal epithelium). A change to a stratified squamous epithelium is referred to as squamous metaplasia.

**Slide 45A: Larynx, H&E and Slide 45B: Larynx, Aldehyde-Fuchsin Masson**

These sections are exactly alike except that different stains have been used. Hold the slide against a light-colored background to orient yourself before beginning your microscopic examination. Identify the large thyroid cartilage and the smaller cricoid cartilage. Recall that the thyroid cartilage is located superior to the cricoid. Identify the two vocal folds. The true vocal fold is inferior to the false vocal fold (ventricular fold or vestibular fold), and both run in an anterior-posterior direction.

Now examine the slide under the microscope. The true vocal fold contains the vocal ligament, which is composed mainly of elastic fibers, and the skeletal muscle fibers of the vocalis muscle. Both will be seen in cross section in these frontal (coronal)
sections of the larynx. The true vocal fold is another area subject to extensive wear and tear, and hence is covered by stratified squamous epithelium. In contrast, the false vocal folds are usually covered by respiratory epithelium. Identify the mixed mucoseroserous laryngeal glands. Are they more common in the true vocal fold or in the false vocal fold? (Answer: In the false vocal folds.) Note the lymphocytic infiltration in one of the folds. Look for plasma cells. Some slides may contain lymphoid nodules. Such lymphoid tissue can often be found in the false folds, the true vocal folds, and anywhere else along the respiratory tract where antigen is present.

Slide 35 (HU Box): Hyaline Cartilage, Human (Trachea), or Slide 46A: Trachea
and
Slide 70 (HU Box): Trachea and Esophagus, c.s., or Slide 46B: Trachea, Newborn

NOTE: In some of these slides the section passed through the cricoid cartilage of the larynx & NOT THE TRACHEA (i.e., the section was cut too high). You can tell if this is the case with your slide because the cricoid cartilage is continuous posteriorly whereas the C-shaped cartilages of the trachea are deficient (i.e., open) posteriorly.

The C-shaped cartilages of the trachea, which serve to maintain the patency of the airway, are composed of hyaline cartilage. The free ends are located posteriorly, and are joined by smooth muscle (the trachealis muscle). Between successive rings along the length of the trachea there is a fibrous connective tissue layer. In several versions of these slides the trachea was not sectioned in a perfectly horizontal plane, and pieces of several cartilage rings are therefore visible, making the section look more like a bronchus than a trachea. Be sure you understand that the tracheal wall contains relatively regular C-shaped cartilages, while the bronchial wall contains multiple irregular plates. Why are the tracheal rings deficient posteriorly rather than anteriorly or laterally? (Answer: The gap in the tracheal rings is located posteriorly to allow a large bolus of food to pass down the esophagus, which lies just posterior to the trachea. On Slide 70 HU, observe this relationship of the trachea to the esophagus.)

Identify the respiratory epithelium lining the tracheal lumen. Recall that it includes ciliated cells and goblet cells. The loose connective tissue layer that lies immediately beneath the epithelium is called the lamina propria. The lamina propria is separated from another connective tissue layer called the submucosa by a layer of elastic fibers (not always visible in H&E). These elastic fibers are characteristic of trachea, since in bronchi & bronchioles smooth muscle would be found in this location rather than elastic fibers. The trachea is also characterized by a thick basement membrane underlying the respiratory epithelium. It may not be evident in all sections.

The submucosa contains the secretory portions of mixed muco-serous glands. These sometimes extend posteriorly through the trachealis muscle into the connective tissue layer called the adventitia, which lies exterior to the cartilage rings. The size of the glands and the number of goblet cells in the respiratory epithelium often increase in tracheas subjected to chronic irritation (e.g., smoking). In addition, the cilia are often lost from the ciliated cells of smokers. The increased mucus production and the difficulty in moving this layer of mucus due to the loss of the cilia contribute to smokers’ cough.

Slide 47A: Primary Bronchus, Trichrome

Most versions of this slide include two primary bronchi and two thin-walled arteries. Identify the multiple, overlapping plates of hyaline cartilage that maintain the
patency of the bronchus. A primary (main) bronchus can also be described as an extrapulmonary rather than an intrapulmonary bronchus since it is not immediately surrounded by lung tissue (alveoli). Extrapulmonary bronchi undergo a gradual transition in the shape of the cartilages in their wall. Near the trachea, primary bronchi contain regular C-shaped cartilages similar to those of the trachea. As the primary bronchi near the lungs, their cartilages become more irregular in shape.

Bronchi are lined by respiratory epithelium, and submucosal glands are still present, but there is now a smooth muscle layer separating the lamina propria from the submucosa instead of elastic fibers. Near the bronchi in this slide, in an area that must be close to the hilus of the lung, are a lymph node, several nerves and two large arteries. Which arteries are this likely to be, and why do they have such a thin wall relative to the size of the lumen? (Answer: They are branches of the pulmonary artery. They have a relatively thin wall because they are part of a low pressure system.)

Primary bronchi divide into lobar (secondary) bronchi (3 for the right lung and 2 for the left) and eventually into segmental (tertiary) bronchi. Each segmental bronchus supplies a bronchopulmonary segment. Bronchopulmonary segments are surgically significant since each has its own blood supply and is separated from its neighbors by connective tissue septa, facilitating removal of the segment as a unit. Segments are too large to be identified on our slides.

Slide 71 (HU Box): Lung, or Slide 48A (Several Versions): Lung

There is a lot of detail in the seemingly random, spongy tissue of the lung. On this slide you will probably be able to identify all the types of airways from bronchi down to alveoli. Specifically you should look for:

Bronchioles:

Bronchioles differ from bronchi in that bronchioles have no cartilage or glands in their walls. A bronchiole can be subclassified as either a conducting bronchiole or a respiratory bronchiole and you should be able to distinguish between the two.

Find a conducting bronchiole. These are lined by an epithelium that changes from the respiratory epithelium (pseudostratified columnar) in the largest conducting bronchioles to a simple columnar or cuboidal epithelium as the diameter of the bronchiole decreases. This simple epithelium is often referred to as “bronchiolar epithelium”. Bronchiolar epithelium is characterized by a new cell type called the Clara cell (bronchiolar cell). The number of Clara cells increases steadily, while ciliated cells and goblet cells become less frequent. Conducting bronchioles also have a relatively thick smooth muscle layer. In asthma attacks the restriction of air flow is largely due to constriction of smooth muscle in the bronchiolar walls, because there is no cartilage to help maintain airway patency. The smallest conducting bronchioles are called terminal bronchioles. The epithelium of a terminal bronchiole is low cuboidal with ciliated cells and Clara cells. It usually lacks goblet cells. A terminal bronchiole by definition bifurcates to give rise to two or more respiratory bronchioles. You have to be very lucky to find this branching point in a section, so you will probably not find a terminal bronchiole. Wheater has an excellent illustration in Fig. 12.12, p. 230.

Respiratory bronchioles are identified by the fact that their lumen is lined in some places by bronchiolar epithelium and in others by alveolar epithelium (which has a squamous appearance due to the presence of squamous type 1 alveolar cells). Because efficient gas exchange can occur across this thin epithelium, respiratory bronchioles are part of the respiratory portion of the lung rather than the conducting portion.
A word or two more needs to be said about Clara cells. In most of our slides Clara cells are not readily identifiable. One of their morphological characteristics is that they tend to bulge into the airway lumen (Ross, Fig. 19.13, p. 678). Their apical ends are either dome-shaped or pointed like the tip of a candle flame, hence their old name of “flame cells”. Clara cells secrete some but not all of the protein components of surfactant. Only the alveolar type II cells can secrete all the constituents of surfactant (lipids as well as proteins). Clara cells also secrete Clara cell protein (CC16) into the airway. This substance is used as a pulmonary marker. When CC16 is lower than normal in bronchoalveolar lavage fluid it indicates damage to Clara cells (hence possible lung injury). Increased levels of CC16 in the serum indicate leakage of the air-blood barrier. Clara cells are not normally associated with nerve terminals.

Several minor cell types are also present in respiratory and bronchiolar epithelium, but are usually impossible to identify in ordinary LM sections. One example is the “small granule cell” or Kulchitsky cell (Wheater, Fig. 12.11b, p. 230), which is part of the diffuse neuroendocrine system (DNES). Unlike Clara cells, Kulchitsky cells have secretory granules at the basal end of the cell rather than the apical end, and hence secrete into the lamina propria rather than into the airway. These granules contain a variety of hormones such as bombesin, calcitonin or serotonin. Some Kulchitsky cells are associated with nerve terminals and are thought to participate in respiratory reflexes that regulate the diameter of the airways or blood vessels. Other types of DNES cells secreting different hormones are found in the epithelium of the GI and urogenital tracts.

**Alveolar Ducts, Alveolar Sacs & Alveoli:**

Respiratory bronchioles lead into alveolar ducts. These are tubular airways whose wall is composed only of alveoli (i.e., only alveolar epithelium, no bronchiolar epithelium). Alveolar ducts often end in spherical enlargements called alveolar sacs. An alveolar sac is composed of several alveoli that open onto a common central space sometimes called the atrium.

In an alveolus, identify alveolar macrophages (dust cells). These often contain visible phagocytized material, especially in the lungs of smokers. It may be possible at high power to identify type II alveolar cells (type II pneumocytes, great alveolar cells, septal cells). These are rounded cells that are often located at the angle formed where interalveolar septa meet. The cytoplasm of the type II cells often appears foamy or vacuolated. What causes this? (Answer: This appearance is due to the fact that the surfactant contained within the lamellar bodies in the cytoplasm of type II cells is usually extracted during tissue preparation, leaving much of the cytoplasm empty-looking or “foamy”). Most of the surface area of the alveolus is covered by type I alveolar cells (squamous alveolar cells, type I pneumocytes), hence the alveolar epithelium is usually classified as a simple squamous epithelium even though it does contain the rounded type II cells. Type I cells are usually so thin that only their nuclei are visible. You must be careful to distinguish type I cells from the endothelial cells lining the capillaries in the alveolar septum. Identify the extensive capillary plexus in the alveolar septa.

**Pulmonary Vasculature:**

Identify branches of the pulmonary artery and vein. The branches of the pulmonary artery tend to run close to the bronchi and bronchioles, while the branches of the pulmonary vein lie further from the large airways, often in the connective tissue septa between bronchopulmonary segments. Try to find a bronchiolar artery. These lie in the wall of the bronchi and bronchioles, but are much smaller than the pulmonary artery accompanying the same airway. Do the bronchiolar arteries carry blood that is oxygenated or deoxygenated? Why are they so much smaller than the pulmonary artery that accompanies the same airway? (Answer: Bronchiolar arteries carry oxygenated blood that supplies the tissues in the wall of the airway. They are small compared to the
nearby pulmonary artery because they are closer to the capillary bed that they ultimately supply. That is, their capillary bed is in the wall of the bronchus or bronchiole itself, while the pulmonary artery still has quite a distance to travel before it reaches the alveolar capillaries that it supplies.)

Mesothelium:

In sections that include the surface of the lung, look for the mesothelium, which is the outer layer of the visceral pleura.

Slide 72 (HU Box): Lung, Elastic Stain, or Slide 48B: Lung, Elastic Fiber

Note the relative abundance of elastic tissue in the walls of large airways and blood vessels. Elastic fibers also encircle the mouths of alveoli that are associated with alveolar sacs (much like the drawstring on a laundry bag). Thus they are often seen in the knobs at the ends of interalveolar septa. Alveoli associated with alveolar ducts are more likely to have smooth muscle cells encircling their mouths. In some forms of emphysema elastic fibers break down and the septa begin to resorb, resulting in decreased total surface area for gas exchange. Excessive resorption can mean that the blood may not become fully oxygenated as it passes through the alveolar capillaries.

Slide 41 (HU Box): Fetus, Rat, Sagittal section

Examine your section of slide 41 to see if it passes through the lungs. If so, note that this is an early stage of lung development (called the pseudoglandular stage), when the lung has a rather solid appearance that resembles an exocrine gland. For the most part only the larger airways have developed, and they resemble the ducts of a gland. Look around the slide and identify as many other organs and structures as you can.

Slide 19 (HU Box): Lung, Fetal

Briefly examine this slide from a later period of lung development (the canalicular stage). The walls of the largest bronchioles now contain a smooth muscle layer and have ciliated cells in the epithelium. Many smaller airways lined by a simple cuboidal to columnar epithelium are present, but type I cells have not yet begun to differentiate. More capillaries are present than would be found in the pseudoglandular period, but efficient gas exchange still could not occur across the walls of these airways.

BRONCHIOLE & ALVEOLAR DUCT IDENTIFICATION ON THE LASERDISK

The faculty disagrees with the identification of the different types of bronchioles presented on p. 117 of the barcode notebook. Listed below are the barcodes in question, the identification given in the barcode notebook, and what we feel that structure really is.

#22038 is listed as “Terminal bronchiole.” We feel that this airway is a respiratory bronchiole (on the right) that is becoming an alveolar duct (on the left).

#21966 and #22041 are listed as “Respiratory bronchioles”. We feel that they are alveolar ducts.

#22221 is listed as “Respiratory bronchiole”. The magnification is too low to be positive, but it looks more like an alveolar duct on the left (the few dark-staining patches are
probably smooth muscle cells, fibroblasts and elastic fibers encircling the mouths of the alveoli), and a respiratory bronchiole on the right.

Remember the criteria for making these identifications:
1. An alveolar duct has its wall lined entirely by alveolar epithelium (type I & type II cells), so by LM it will appear to have a simple squamous epithelium because type I cells cover most of the surface area.
2. A respiratory bronchiole by definition must have part of its wall lined by alveolar epithelium and part lined by bronchiolar epithelium (simple cuboidal or columnar), which will stain darker than alveolar epithelium.
3. A terminal bronchiole has its wall entirely lined by bronchiolar epithelium and divides to give rise to respiratory bronchioles. To be sure that an airway is a terminal bronchiole you must see the branching. Otherwise all you can say is that the airway that is lined entirely by bronchiolar epithelium is a conducting bronchiole (i.e., it has no alveolar epithelium in its walls).

ELECTRON MICROSCOPY (RHODIN)
1. Note that by EM it is easier to distinguish the three cell types of the olfactory epithelium (Figs. 31-4 & 31-5) than by LM. Identify the:
   - Sustentacular (supporting) cells with many long, thin, branching microvilli (Figs. 31-5 & 31-6). Their nuclei tend to be in the layer closest to the surface of the nasal cavity.
   - Basal cells with their nuclei located near the basement membrane of the epithelium (Fig. 31-4). These are the stem cells for this epithelium.
   - Olfactory receptor cells with their nuclei at a level between those of the basal cells and the sustentacular cells. Olfactory cells are true bipolar neurons. Their apical end (Fig. 31-7 & 31-8) is a bulb-like enlargement called the olfactory vesicle. From the olfactory vesicle several extremely long cilia extend outward to lie parallel to the surface of the epithelium. These cilia have odor receptors in their plasma membrane. The basal end of the olfactory cell (not shown in these micrographs) gives rise to an axon that eventually joins the olfactory nerve.
2. Study “the respiratory epithelium” (Fig. 31-22) and know in which types of airways it is found. Identify ciliated cells and the goblet cells. Note that Fig. 31-3 shows the transition from olfactory epithelium on the right to respiratory epithelium on the left.
3. In the bronchioles note the Clara cells (Figs. 31-39 & 31-40). They are nonciliated, and often protrude further into the airway than the ciliated cells. Clara cells have apical secretory vacuoles containing glycoproteins that they secrete into the airway. They produce some of the proteins and phospholipids of surfactant, but not the main surface tension-lowering component. In addition they metabolize certain pollutants, and serve as the stem cell for bronchiolar epithelium.
4. Be aware that other rarer epithelial cell types not illustrated in Rhodin also exist. These include the brush cell and small granule cell (Kulchitsky or K cell, Wheater, Fig. 12.11b, p. 230). Brush cells are in synaptic contact with nerve terminals and are thought to be receptor cells of some type although they do not play a role in olfaction. Small granule cells are endocrine cells that produce mediators such as serotonin and bombesin.
5. Observe the epithelium of a respiratory bronchiole by LM (Fig. 31-42) & EM (Fig. 31-43). Notice how the simple cuboidal epithelium is abruptly interrupted by an alveolus lined mainly by squamous alveolar type 1 cells. The presence of these two types of epithelia lining the airway defines it as a respiratory bronchiole. In Fig. 31-43 note the close association between the alveolar epithelium and the capillaries, allowing efficient gas exchange.

6. Study the features of the alveolar walls, including pulmonary capillaries (Figs. 31-46 & 31-55), and type I alveolar cells (Fig. 31-55), type II alveolar cells (Figs. 31-47 to 31-50). Identify the components of the minimum air-blood barrier separating the capillary lumen from the lumen of the alveolus (Fig. 31-55 & 31-56). These are the type I cell cytoplasm, the fused basal laminae, and the endothelial cell cytoplasm. Where would the surfactant layer be located? [Answer: In the alveolar airspace (labeled “1” in these figures), where it floats on an aqueous hypophase. Since it is lipid, surfactant is often extracted during tissue preparation unless special precautions are taken.]

LABORATORY 14 CHECKLIST
RESPIRATORY SYSTEM

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<thead>
<tr>
<th>LIGHT MICROSCOPY</th>
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<tr>
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<td>Clara cell</td>
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<td>olfactory epithelium</td>
<td>type I pneumocyte</td>
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<td>sustentacular cells of olfactory epithelium</td>
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<td>basal cells of olfactory epithelium</td>
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<td>bronchiolar 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 14: RESPIRATORY 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. Describe what is meant by the term “respiratory epithelium”? In which types of airways would you normally expect to find it?

2. Different types of epithelium line different parts of the respiratory tract because each epithelium performs some specialized function that is required in that type of airway. What are the functions of the different types of epithelium found in the respiratory tract?

3. Characterize the pulmonary arteries, bronchiolar arteries, and pulmonary veins, in terms of the oxygen content of the blood they carry.

4. Why aren’t pulmonary capillaries fenestrated to make them more permeable?

5. What structural features help minimize the thickness of the minimal air-blood barrier?

6. What is meant by the term “mucociliary escalator”?

7. How is particulate matter removed from the alveoli?

8. What function do pores of Kohn serve?

9. In a high magnification light micrograph of an airway that showed only the mucosa and part of the submucosa, what characteristics would tell you for certain that this was the trachea?

10. What morphological characteristics would you use to distinguish between the following: respiratory bronchiole, terminal bronchiole, and alveolar duct?

11. In asthma, most of the impediment to air flow occurs at the level of the bronchiole. What structural features of a bronchiole help explain why this is true?

12. How would you distinguish the olfactory epithelium from the respiratory epithelium? Where would you find the olfactory epithelium?

13. Describe the olfactory nerve (cranial nerve I).

14. How do you think that laryngitis changes the quality of your voice?
LABORATORY 15

ORAL CAVITY & UPPER GI TRACT

I. ORAL CAVITY & TEETH

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

1. identify the various types of papillae and glands associated with the tongue: filiform, fungiform, foliate and vallate (circumvallate)
2. describe the structure of a taste bud
3. distinguish between the three salivary glands: parotid, sublingual and submandibular
4. identify the parts of a tooth: crown, neck, and root
5. locate the following tissue types within a tooth:
   - enamel
   - dentin
   - cementum
   - pulp
6. recognize the following parts of a developing tooth:
   - enamel organ (including outer enamel epithelium, stellate reticulum, stratum intermedium, ameloblasts, and enamel)
   - dental papilla (including dentin, predentin, & odontoblasts)
   - dental lamina
   - dental sac
7. understand why enamel production ceases at the time of tooth eruption whereas dentin normally continues to be laid down as long as a tooth remains viable
8. distinguish between the three types of tonsils: lingual, pharyngeal and palatine

LABORATORY:

Please study the following slides in your set:

A. Vallate & Filiform Papillae; Taste Buds
Slide 11 (HU Box): Vallate Papillae
Slide 56A: Taste Buds, or
Slide 56B: Taste Buds, Trichrome
Identify a vallate (circumvallate) papilla. It is the largest type of papilla and is characterized by:
- a deep moat-like crypt surrounding the papilla
- numerous taste buds embedded in the walls of this moat
- large serous glands (von Ebner’s glands) that empty into the base of the moat
Vallate papillae lie in a single row just anterior to the v-shaped sulcus terminalis that divides the tongue into an anterior 2/3 and a posterior 1/3. There are roughly 8-12 vallate papillae in humans. They are separated from one another by considerable distances. This helps to distinguish them from foliate papillae, which lie directly next to one another. Identify the filiform papillae. These are the most common type and the smallest. They have a characteristic pointed shape and carry no taste buds.

Observe the taste buds of the vallate papillae. Each contains several nerve fibers that mediate the sensation of taste. These fibers are associated with the neuroepithelial (sensory) cells of the taste bud. In addition the taste bud contains supporting or sustentacular cells, and a population of basal cells, which are believed to be stem cells for both the neuroepithelial and sustentacular cells. It is not necessary to distinguish between the neuroepithelial and sustentacular cells, both of which are elongated in shape. However, the rounder basal cells may be identifiable. Taste buds are almost completely covered by the stratified squamous epithelium of the tongue, except for a small opening called the taste pore. (Wheater, Fig. 21.1b, p. 402).

Study the skeletal muscle bundles making up the intrinsic muscles of the tongue. Notice that they are oriented in three mutually perpendicular directions (vertical, horizontal and longitudinal). This arrangement is diagnostic for tongue.

B. Foliate papillae

Slide 55 (HU Box): Tongue

Some versions of this slide show foliate papillae, which actually are a series of vertically oriented ridges rather than individual cylindrical or fungiform structures. They are located on the posterolateral sides of the tongue. In sections where the adjacent ridges are cut in cross section, you will see what looks like a row of papillae lying immediately next to one another with no other structures intervening between them. This distinguishes them from vallate and fungiform papillae, which are widely separated from one another. Foliate papillae carry taste buds that are mainly located on their lateral surfaces. In humans, these taste buds decrease in number with age, and the foliate papillae themselves become somewhat less prominent.

There are no examples on your slides of fungiform papillae. Fungiform papillae carry taste buds (usually on their dorsal surface), and are scattered over the dorsum of the anterior 2/3 of the tongue (see Wheater, Fig. 13.13, p. 247).

C. Salivary Glands

Slide 12 (HU Box): Parotid Gland, and
Slide 13 (HU Box): Salivary Gland, Sublingual, Human, or
Slide 57B: Sublingual Gland
and
Slide 14 (HU Box): Strat. Column. Epithel., Human (Submandibular Gland), or
Slide 57C: Submandibular Gland

Study and compare the three major salivary glands. To distinguish between them, decide whether the gland contains only serous cells (parotid), or a mixture of serous and mucous cells. If a mixture is present, determine whether the mucous cells predominate with serous cells present mainly as serous demilunes (sublingual), or whether the balance between serous and mucous cells is more equal, with serous cells present as pure serous acini as well in demilunes (submandibular).
Be sure you can distinguish secretory acini from the various types of intralobular ducts including striated ducts and intercalated. In the parotid gland, also look for the adipose tissue that tends to increase with age.

**Slide 96 (HU Box): Salivary Gland Complex, Rat**

The rat possesses a unique arrangement of salivary glands at the angle of the jaw that includes the parotid, submandibular and sublingual glands. Depending on how this tissue complex was sectioned, you should find at least two of these three glands. Embedded in the connective tissue supporting the complex are isolated lymph nodes. Do not mistake these for salivary gland acini.

**D. Teeth**

**Slide 58: Development of Tooth**

**Slide 58A: Tooth Formation, Infant, and**

**Slide 58B: Tooth Development, Late Stage of Dentin**

These slides all show stages in the development of the crown of a tooth. None of them show the root of a tooth. Find the components of the enamel organ: outer enamel epithelium, stellate reticulum, and inner enamel epithelium (this has differentiated into ameloblasts in many locations). What hard layer of the tooth do ameloblasts produce? *(Answer: Enamel)*

Identify odontoblasts and the dental papilla. Odontoblasts differentiate from the mesenchymal cells of the dental papilla, which are of neural crest origin. Odontoblasts produce predentin, which then becomes mineralized to form dentin. Notice that the ameloblasts are located on the outer surface of the tooth, whereas odontoblasts line the inner surface next to the dental papilla. As the ameloblasts and odontoblasts lay down enamel and dentin respectively, the two cellular layers are gradually pushed further and further apart. Ameloblasts can usually be distinguished from odontoblasts in a high magnification light micrograph because they form a “neater”, more-orderly looking layer of cells than the odontoblasts.

Development of the tooth proceeds from the crown downward towards the neck and finally the root of the tooth. Follow the layers of enamel and predentin/dentin down toward the neck of the tooth on slide 58 or 58A and note that you eventually come to a point where predentin/dentin is present, but there is no enamel yet. This is because odontoblasts begin to produce predentin/dentin before ameloblasts produce enamel (even though ameloblasts differentiate before odontoblasts do).

**E. Tonsils**

**Slide 10 (HU Box): Tonsil, Palatine, Human, or**

**Slide 22: Tonsil (Faucial)**

*(NOTE: All versions of Slide 22 are palatine tonsil unless labeled “Pharyngeal”.*

The faucial or palatine tonsil is a group of lymphatic nodules lying in the tonsillar fossa on each side of the oropharynx. These are the tonsils you can see when you stick out your tongue and say “Ahhhh.” They are characterized by a surface epithelium which is minimally keratinized stratified squamous, and which invaginates to form numerous deep tonsillar crypts (see Wheater, Fig. 11.15, p. 216). Beneath the epithelium many lymphoid nodules line up along the crypts. In any type of tonsil, it is common for lymphocytic infiltration of the epithelium to be so heavy in places that it is difficult to identify the epithelial cells.
Slide 9 (HU Box): Pharyngeal Tonsil, Human, or 
Slide 22: Tonsil, Pharyngeal

When enlarged and inflamed, the pharyngeal tonsil is commonly called adenoids ("adeno" means gland; "oid" means like). Although it is difficult to see in this section due to heavy infiltration of the epithelium by lymphocytes, the pharyngeal tonsil is covered for the most part by pseudostratified ciliated columnar epithelium (the respiratory epithelium), with some patches of stratified squamous epithelium as well. The pharyngeal tonsil lacks crypts but has instead a folding (plication) of the surface epithelium. Although this distinction is readily apparent when viewed by SEM, to distinguish between crypts and folds in sectioned material you would have to examine serial sections. The unpaired pharyngeal tonsil lies in the posterior-superior wall of the nasopharynx. Enlargement may lead to partial obstruction of the choanae (the openings from the nasopharynx to the nasal cavity), leading to mouth breathing. Similarly, enlarged pharyngeal tonsils may obstruct the opening of the auditory tube into the nasopharynx, leading to ear infections.

Our slide sets include no examples of the lingual tonsils. A lingual tonsil can be identified by the minimally keratinized stratified squamous epithelium that covers it and by the fact that each lingual tonsil has a single short unbranched crypt (Wheater, Fig. 13.13, p.247). They are embedded in the posterior third of the dorsum of the tongue.

II. ESOPHAGUS

OBJECTIVES

1. name and identify the layers of the esophageal wall
2. distinguish between cross and longitudinal sections based on the orientation of the muscle layers
3. distinguish esophagus from stomach
4. describe the specific structural variations that allow you to distinguish between the upper, middle and lower regions of the esophagus

LABORATORY:

Please study the following slides in your set:

Slide 56 (HU Box): Esophagus, c.s., or
Slide 60: Esophagus

In these cross sections of the esophagus, note the four-layers that make up the wall of the organ: mucosa, submucosa, muscularis externa and adventitia. This same arrangement was seen in the airways of the respiratory tract, and is typical of many of the hollow viscera of the body.

The mucosa of the esophagus includes: the epithelium, the lamina propria (a connective tissue layer), and the muscularis mucosae (a layer of smooth muscle that separates lamina propria from submucosa). The epithelium is a minimally keratinized stratified squamous epithelium. This epithelium allows you to distinguish esophagus from stomach and intestines, which are lined by simple columnar epithelia.
Examine the muscularis externa. This is composed of an inner circular layer and an outer longitudinal layer. The type of muscle present in the muscularis externa changes as you move down the organ toward the stomach. Which end of the esophagus do you think will have smooth muscle in its muscularis externa and which will have skeletal? Why? (Answer: The upper portion has skeletal muscle in the muscularis externa, while the lower portion has smooth. This makes sense because the upper end is continuous with the pharynx whose walls contain skeletal muscle, while the lower end is continuous with the stomach, which contains only smooth muscle. The midsection of the esophagus has a mixture of skeletal and smooth muscle in the muscularis externa.)

The wall of the esophagus contains scattered mucous glands, which can be located either in the lamina propria (esophageal cardiac glands) or in the submucosa (esophageal glands proper). Esophageal cardiac glands are more common at the upper and lower ends of the esophagus, whereas esophageal glands proper can be found in the submucosa anywhere along the length of the esophagus. Decide whether your sections were taken from the upper, middle, or lower portion of the esophagus by noting the kinds of muscle in the muscularis externa and the location of any glands that may be present.

III. STOMACH

OBJECTIVES:
1. describe the specific structural variations that allow you to distinguish between:
   - esophagus and stomach
   - cardiac, fundic and pyloric regions of the stomach
2. identify and explain the function(s) of the epithelial cell types of the stomach, including:
   - surface mucous cells
   - mucous neck cells
   - parietal cells
   - chief cells
   - enteroendocrine cells
3. understand the relationship between gastric pit and gastric glands and be able to identify each in all regions of stomach
4. indicate the location of the stem cell population in the gastric epithelium
5. describe and identify a ruga

LABORATORY

Please study the following slides in your set:

Slide 15 (HU Box): Esophagus and Stomach, l.s., or Slide 60A: Cardio-Esophageal Junction
and
Slide 57 (HU Box): Stomach, Cardiac Region
Slides 15 (HU) and 60A are longitudinal sections through the gastro-esophageal (cardio-esophageal) junction, i.e., the lower end of the esophagus and the first portion (cardiac region) of the stomach (see Wheater Fig. 14.6, p. 256). What type of epithelium lines each organ? (Answer: Minimally keratinized stratified squamous epithelium lines the esophagus, and simple columnar epithelium lines the stomach.) Notice that there is quite an abrupt change from one type of epithelium to the other at this cardio-esophageal junction rather than a gradual thinning down to one layer.

In these slides and in slide 57(HU) study the cardiac region of the stomach. Locate the gastric pits and the cardiac glands. A gastric pit is a tubular invagination of the surface epithelium. In all regions of the stomach several glands empty into the base of a single pit. Both gastric pits and gastric glands are located in the lamina propria. Identify the surface mucous cells that line the lumen of the stomach and the gastric pits. The surface mucous cells usually have their mucous granules limited to the apical region of the cytoplasm, forming what is sometimes referred to as an apical mucus cup. The nucleus remains uncompressed in the basal region of the cell. Cardiac gland cells also produce mucus, but have a different morphology in that they lack such an obvious apical cup. Enteroendocrine cells are also present in the surface epithelium, pits and glands but are difficult to identify in cardiac stomach since they are light-staining like the other cell types present here.

Where are the epithelial stem cells located in the stomach? (Answer: In the neck of the glands) Look for mitotic figures in that region. The same stem cell population gives rise to the surface mucous cells, enteroendocrine cells and gastric gland cells. That means that some differentiating cells move up toward the surface of the stomach and others move down into the glands in a well-orchestrated procession.

Identify the lamina propria, muscularis mucosae, submucosa and muscularis externa in esophagus and stomach. Note elements of the myenteric plexus between layers of the muscularis externa. On some slides the mesothelium of the serosa of the stomach is visible. On others the entire serosa may have been torn off.

Slide 58 (HU Box): Stomach, Fundic Region, Human, or Slide 61A: Fundic Stomach

The fundic stomach is characterized by long gastric glands that contain parietal cells and chief cells. Why do parietal cells have an intensely eosinophilic cytoplasm? (Answer: Because they have so many mitochondria. The many proteins in the membranes of a mitochondrion (cytochromes, etc.) cause it to be extremely eosinophilic.) What accounts for the basal basophilia of the chief cells? (Answer: The RNA in the ribosomes of their extensive RER. These cells secrete large amounts of enzymes including pepsinogen and, in humans, lipase)

If possible identify an enteroendocrine cell. These cells have a pale cytoplasm and are usually scattered singly among the other cell types of the epithelium. They may be in the surface epithelium, in the pits or in the fundic glands. They are easiest to identify in the glands where their pale cytoplasm contrasts with the eosinophilia of parietal cells and the basophilia of chief cells and makes them easier to see. In what part of the cytoplasm are the secretory granules of enteroendocrine cells localized? Why? (Answer: Enteroendocrine cells have secretory granules at their basal ends because they secrete into the connective tissue surrounding the glandular epithelium, rather than into the lumen. They do this because they are endocrine cells. Their hormonal products either affect target cells in the immediate vicinity or are picked up by blood vessels and carried to distant target organs.)
See if you can confirm the fact that several glands open into the base of a single pit. This is true for all regions of the stomach, but is usually easiest to see in the fundic region. Be aware that the morphology described here as fundic stomach is found in what the gross anatomist would call the body of the stomach as well as in the fundus.

Rugae are temporary folds that form in the relatively empty stomach and disappear as the stomach fills and distends. What layers contribute to the formation of a ruga? \(\text{Answer: Mucosa and submucosa}\)

On any of the stomach slides look for the muscularis externa. It is traditionally described as having three layers: outer longitudinal, middle circular and inner oblique, but the arrangement is actually much more complex than that, and differs in different regions and on different surfaces of the stomach. This less regular arrangement does however help to distinguish stomach from small or large intestine where the outer longitudinal and inner circular layers of the muscularis externa are consistently present.

Slide 59 (HU Box): Stomach, Pyloric Region, Human, or Slide 62: Stomach, Pyloric

In the pyloric stomach the gastric glands are composed of mucous cells and enteroendocrine cells, which again are difficult to identify. The glands resemble those of the cardiac region. However, pyloric stomach can be distinguished from cardiac because it has extremely deep pits. Notice that in all three histological regions of the stomach the surface mucous cells have the same morphology.

IV. ELECTRON MICROSCOPY

A. ORAL CAVITY & SALIVARY GLANDS

Identify the following:

1. The various types of papillae on the tongue:
   - Filiform (Fig. 26-13 to 26-15): carry no taste buds.
   - Fungiform (Fig. 26-19): taste buds on dorsal surface; are scattered as single papillae among the filiform.
   - Circumvallate (vallate) (Fig. 26-20): large size, deep moat and taste buds on lateral walls of papilla and moat
   - Foliate (Fig. 26-21) (rare or absent in adult humans): formed by folds on the lateral surface of the tongue; when the folds are sectioned transversely, the papillae appear to be in rows right next to one another.

2. Taste buds (Fig. 26-23): identify the taste pore and the light and dark cells. Light and dark cells extend the full length of the taste bud from its base to its taste pore. At least one good example of the rounder, shorter basal cells (#6) is shown at the lower border of the taste bud.

3. Serous vs. mucous cells of the salivary glands (Figs. 27-3 vs. 27-8 & 27-9) and serous demilunes (Figs. 27-8 & 27-9). The serous cells of the demilune secrete into very narrow channels between mucous cells that empty into the lumen of the acinus.

4. Intercalated ducts (Fig. 27-10) and striated ducts (Fig. 27-11, 27-12 & 27-15) of salivary glands. Since, in salivary glands, the intercalated
ducts and striated ducts are normally found within the lobules, they both represent types of intralobular ducts in these glands.

5. In the ground sections of a mature tooth (Figs. 27-16, 27-17 & 27-19) identify crown, neck, root, pulp cavity, enamel, dentin, and cementum. In Fig. 27-20 notice that cementoblasts can become entirely surrounded by the matrix they secrete, occupying lacunae in the cementum. In demineralized sections of a mature tooth also identify the location of the periodontal ligament (called periodontal membrane in Rhodin) and the bony socket of the tooth (Figs. 27-21 and 27-25). The bundles of collagen fibers that make up the periodontal ligament are anchored at one end into the bone matrix and at the other end in the cementum of the tooth root. These entrapped bundles are referred to as Sharpey’s fibers. Notice that in decalcified sections the enamel is usually missing because mature enamel consists mainly of inorganic salts that are removed by the decalcification process.

6. In the developing tooth (Figs. 27-27 to 27-30) identify the stellate reticulum, stratum intermedium, ameloblasts, enamel, dentin, predentin, odontoblasts, and dental papilla. The stellate reticulum eventually collapses on itself so that the outer enamel epithelium approaches the ameloblasts. As it does so, the stellate reticulum forms a compressed layer up against the ameloblasts. This layer of mesenchymal cells is called the stratum intermedium.

B. STOMACH

Identify the following:

1. Surface mucous cells: Observe that the mucous granules which are usually lost during tissue processing for LM (Fig. 28-10) are often preserved in samples prepared for EM (Figs. 28-11 & 28-12).

2. In the light micrograph shown in Fig. 28-10 identify the gastric pit (#2), a gastric gland (#3), and the neck of the glands (#4) where the stem cells are located.

3. In the fundic glands (called gastric glands proper in the figure captions) identify the two main cell types: chief cells and parietal cells (Figs. 28-15 & 28-16).

4. In a high power view (Fig. 28-17) observe that chief cells are typical protein secretors, characterized by well-developed RER and Golgi and by numerous secretory vacuoles. Parietal cells are ion pumps that contain numerous mitochondria, and also intracellular canaliculi lined by microvilli. These canaliculi are a mechanism for increasing the apical membrane surface area to make room for more ion pumps. The parietal cell secretes hydrogen and chloride ions into the lumen of the stomach. Note the extensive system of tubular and vesicular membranes in the parietal cell cytoplasm adjacent to the canaliculi. These can rapidly fuse with the plasma membrane to increase its surface area even further. Compare the parietal cell with the oxyphil of the parathyroid gland, which also contains many mitochondria.

5. Mucous gland cells & enteroendocrine cells (Figs. 28-20 & 28-21). Note that the secretory granules of the enteroendocrine cells are stored in the basal rather than the apical end of the cytoplasm since
they are released across the basal plasma membrane and not into the lumen. The secretory granules of the mucous gland cells are apically located near the lumen.

**LABORATORY 15 CHECKLIST**  
**ORAL CAVITY & UPPER GI TRACT**

<table>
<thead>
<tr>
<th>LIGHT MICROSCOPY</th>
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<tbody>
<tr>
<td>circumvallate (vallate) papilla</td>
<td>submucosa of esophagus &amp; stomach</td>
</tr>
<tr>
<td>taste bud</td>
<td>adventitia of esophagus</td>
</tr>
<tr>
<td>von Ebner’s gland</td>
<td>serosa of stomach</td>
</tr>
<tr>
<td>filiform papilla</td>
<td>upper vs. middle vs. lower esophagus</td>
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<tr>
<td>foliate papilla</td>
<td>esophageal cardiac glands</td>
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<tr>
<td>striated duct</td>
<td>esophageal glands proper</td>
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<tr>
<td>parotid vs. submandibular vs. sublingual gland</td>
<td>muscularis externa of esophagus &amp; stomach</td>
</tr>
<tr>
<td>3 parts of the enamel organ</td>
<td>cardio-esophageal junction</td>
</tr>
<tr>
<td>ameloblast</td>
<td>surface mucous cells of stomach</td>
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<tr>
<td>enamel</td>
<td>gastric pits vs. gastric glands</td>
</tr>
<tr>
<td>predentin</td>
<td>cardiac vs. fundic vs. pyloric stomach</td>
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<tr>
<td>dentin</td>
<td>location of epithelial stem cells in stomach</td>
</tr>
<tr>
<td>odontoblast</td>
<td>myenteric plexus of stomach</td>
</tr>
<tr>
<td>dental papilla</td>
<td>parietal cells</td>
</tr>
<tr>
<td>cementum</td>
<td>chief cells</td>
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<tr>
<td>periodontal ligament</td>
<td>enteroendocrine cells</td>
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<tr>
<td>palatine vs. pharyngeal vs. lingual tonsil</td>
<td>rugae</td>
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<td>mucosa of esophagus &amp; stomach</td>
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<tr>
<th>ELECTRON MICROGRAPHS</th>
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<tr>
<td>taste bud</td>
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<td>serous vs. mucous gland cell</td>
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<td>stellate reticulum</td>
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<td>chief cell</td>
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<tr>
<td>enteroendocrine cell</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 15: ORAL CAVITY & UPPER GI TRACT

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. Older individuals sometimes report that their mouth feels very dry. This is because the volume of saliva that is produced decreases gradually with age. What change occurs in the morphology of the aging parotid gland that could contribute to a decrease in secretory volume?

2. What is the embryological origin of the cells that produce tooth enamel? Of the cells that produce predentin and dentin?

3. Why do we have to visit the dentist if we have a serious defect in the enamel (i.e., a cavity) in one of our teeth? Why can’t teeth produce more enamel to repair the defect?

4. How would you distinguish between the upper, lower, and middle portion of the esophagus?

5. Why are the esophageal glands that are located in the lamina propria called esophageal cardiac glands?

6. What is the morphological difference between a serosa and an adventitia?

7. Intraperitoneal organs are suspended directly or indirectly from the body wall by a mesentery. What is the function of a mesentery other than to keep our intraperitoneal organs from sloshing around randomly? Describe the histology of a mesentery.

8. What is the structural relationship between a gastric pit and a gastric gland?

9. How can you distinguish the cardiac stomach from the fundic or pyloric stomach?

10. What are the functions of parietal cells and chief cells?

11. Oxyphils (parathyroid gland) and parietal cells (fundic stomach) both contain unusually large numbers of mitochondria. How could you distinguish between them in a medium magnification electron micrograph?
LABORATORY 16

LOWER GI TRACT

OBJECTIVES:

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

1. describe the structural variations that allow you to distinguish between:
   - small intestine and large intestine
   - the parts of the small intestine - duodenum, jejunum and ileum

2. identify and explain the function(s) of intestinal epithelial cell types including:
   - absorptive cells (enterocytes)
   - goblet cells
   - Paneth cells
   - enteroendocrine cells

3. describe where plicae circulares, villi & microvilli are found, and what layers are involved in forming each structure

4. identify the location of the epithelial stem cells in small intestine and large intestine

5. explain the structure and function of a lacteal and find an example of one in a section

6. identify the submucosal plexus and the myenteric plexus. Look for ganglion cells in each. Explain the function of the enteric nervous system.

7. describe the structural features you could use to distinguish small and large intestine from esophagus or stomach

8. identify the following components of the GALT:
   - diffuse lymphocytic infiltration
   - individual nodules
   - groups of nodules including those of the appendix, Peyer’s patches and tonsils

LABORATORY:

Please study the following slides in your set:

A. SMALL INTESTINE
   Slide 16 (HU Box): Stomach and Duodenum, l.s.
   Slide 62A: Pyloro-Duodenal Junction, or
   Slide 63: Stomach and Duodenum, LS at Junction

Identify the abrupt epithelial transition between pyloric stomach and duodenum. Note that both organs are lined by a simple columnar epithelium. However, in the stomach essentially all the surface cells are of one type (surface mucous cells), while in the duodenum and the rest of the intestine the surface epithelium contains two major cell types, goblet cells and intestinal absorptive cells.
Identify the pyloric sphincter present at the pyloro-duodenal junction. What type of muscle makes up the sphincter? (Answer: Smooth muscle) Is it longitudinally or circularly arranged? (Answer: All sphincters must be circularly arranged in the wall of the organ or else they would not be able to act as sphincters.) Compare this region to the cardio-esophageal junction where there is a physiological sphincter but not an anatomical one.

Slide 60 (HU Box): Duodenum, c.s.
Identify the villi. Their presence clearly characterizes this section as small intestine. The presence of Brunner's glands in the submucosa clearly identifies it as duodenum. Another clue that this is duodenum comes from examining the organ visible next to it. What organ is this? (Answer: The pancreas. The head of the pancreas is directly surrounded on three sides by the duodenum.) Identify the intestinal absorptive cells and goblet cells, which are the major cells types in the epithelium covering the villi. Scattered enteroendocrine cells may also be present but again are difficult to identify. These same cell types (at varying stages of differentiation) are present in the intestinal glands (crypts of Lieberkühn). These are simple tubular glands formed by the invagination of the surface epithelium into the lamina propria. By scanning electron microscopy they would look like holes scattered between the bases of the villi. The stem cell population in the small and large intestine is located near the base of the intestinal glands. See if you can identify any mitotic figures in the crypts in this or any other slide of small intestine. Often the nuclei of dividing cells are located closer to the lumen of the crypt than the nuclei of other cells, as if they stepped toward the lumen during mitosis and then stepped back into line when they were finished dividing.

Slide 61 (HU Box): Jejunum, or Slide 64 or 64A: Jejunum
Jejunum is usually identified by what it does not have. It is the part of the small intestine that normally contains "no" Brunner's glands (Brunner's glands are typical of duodenum) and "no" Peyer's patches (Peyer's patches are typical of ileum). In some versions of these slides Paneth cells with large eosinophilic cytoplasmic granules can be seen at the base of the intestinal glands. They are particularly numerous in jejunum and ileum.

Slide 62 (HU Box): Ileum, c.s., and Slide 65 (Trichrome) or 65A: Ileum (H&E)
Most, but not all, sections of ileum contain several lymphoid nodules that are part of a Peyer's patch. Usually these tend to be located on the abmesenteric border of the ileum (the side opposite the point where the mesentery attaches to the intestinal wall, but on some of our slides this is not the case. Notice that the normal pattern of intestinal villi tends to be somewhat altered in the region covering a lymphoid nodule in Peyer's patches. Specifically there tend to be no villi immediately overlying the nodules of a Peyer's patch. Instead there is a dome epithelium. What cell type is present on the villi but largely absent from the dome epithelium? (Answer: Goblet cell) A unique cell type located in the dome epithelium is the M cell. M cells transport antigens across the epithelium & release it near lymphocytes and antigen-presenting cells (APCs). Many lymphocytes and APCs often lie within a deep pocket created by an infolding of the M cell's basal plasmalemma. M cells themselves are often difficult to see by LM, but by
looking for lymphocyte nuclei that appear to be clustered within the epithelium you can deduce where an M cell must be present.

Compare the relative abundance of goblet cells vs. absorptive cells in the ileum vs. duodenum. You should find that goblet cells increase in frequency as you move from duodenum through small and large intestine, reflecting the greater need for lubrication as the fecal mass becomes more solid.

Look for plicae circulares (see Wheater, Fig. 14.16b, p.262). These are present in most of the duodenum, well-developed in the jejunum, and present in the proximal half of the ileum. A plica circularis includes mucosa and submucosa, and differs from a ruga in the stomach in that plica is a permanent structure, while a ruga is temporary. In addition, a plica has many villi on its surface. A ruga, being part of the stomach, has no villi. What makes up the core of a villus (see Wheater, Figs. 14.19, p. 265)? (Answer: Only lamina propria – no submucosa)

B. LARGE INTESTINE

Slide 63 (HU Box): Colon, Human, or
Slide 67 or 67A: Colon, Human

Note that the large intestine has no villi. It also has no permanent folds such as the plicae circulares of the small intestine, although temporary folds of the mucosa and submucosa can occur. Identify the intestinal glands. In both small and large intestine these are simple tubular glands lined by a simple epithelium with goblet cells and absorptive cells. In the colon Paneth cells are rare or absent. Goblet cells are even more abundant than in small intestine, sometimes making it difficult to find the intestinal absorptive cells interspersed among them.

Examine the muscularis externa, especially its outer longitudinal layer. Around the full circumference of the colon this layer has three thickened regions called the teniae coli. Between the teniae, the outer longitudinal layer is extremely thin or absent. Decide whether your section shows the teniae coli or the attenuated region of the outer longitudinal layer.

Re-examine a section of cardiac stomach (Slide 15HU, 57HU, or 60A). Note that you can distinguish between colon and cardiac stomach based on the types of cells in the surface epithelium, the number and orientation of the layers in the muscularis externa, whether the glands open into pits or directly onto the surface, and whether the glands are straight or coiled.

On any slide of small or large intestine (or stomach) identify the ganglia and nerves of the enteric nervous system. They are found in the submucosa (submucosal or Meissner’s plexus) and between the two layers of the muscularis externa (myenteric or Auerbach’s plexus.) Recall that these ganglia contain both sensory and motor neurons, and that they can produce coordinated peristalsis even if the normal input from the autonomic nervous system is cut.

Slide 64 (HU Box): Appendix, c.s., or
Slide 66 or 66A: Appendix, c.s

Notice that the structure of the appendix closely resembles that of the colon except that appendix has no teniae coli; instead the 3 teniae meet at the appendix and unite to form a uniformly thick outer longitudinal layer of the muscularis externa.

The appendix normally contains many lymphoid nodules extending from lamina propria into submucosa. You may confuse these with Peyer’s patches in the ileum, but
in the appendix the nodules tend to be more uniformly distributed around the entire circumference of the gut tube. Also, recall that the basic structure of ileum and appendix differ. Ileum is a part of small intestine and therefore has villi whereas the appendix resembles colon and has no villi. Which class of antibody is produced by most of the plasma cells in the gut? (Answer: IgA)

Slide 17 (HU Box): Recto-Anal Junction, l.s.
Study the epithelial transition between rectum and anal canal (see Wheater, Fig. 14-32, p. 273). The rectum is lined by a simple columnar epithelium similar to that of the colon and has typical straight unbranched intestinal glands. In the anal canal the epithelium changes from a simple epithelium to a stratified epithelium, ending up as a minimally keratinized stratified squamous epithelium in the lower part of the canal. The anal canal also has branched tubular glands that open via ducts onto the anal surface. Follow the anal canal and look for another epithelial transition to the maximally keratinized stratified squamous epithelium of the perianal skin. In the transition zone between anal canal and the perianal region you may see apocrine glands with their characteristically wide lumen.

Compare recto-anal junction with previously studied transition zones:
- Gastro-esophageal junction (Slide 15HU, or 60A)
- Gastro-duodenal junction (Slide 16HU, 62A or 63)

Be able to distinguish one transition zone from another.

C. ELECTRON MICROSCOPY (RHODIN)
Identify the following:

1. In the small intestine distinguish between plicae circulares (Figs. 29-1 to 29-3), villi (Figs. 29-1 to 29-5), and microvilli (Figs. 29-5 to 29-7).

2. Study the structure of a villus (Fig. 29-5). Identify the simple columnar epithelium composed of intestinal absorptive cells, goblet cells and occasional enteroendocrine cells. The core of the villus is the loose cellular connective tissue of the lamina propria. It contains blood vessels, lacteals, and smooth muscle cells arranged parallel to the long axis of the villus.

3. Study the ultrastructure of the following cell types:
   - Intestinal absorptive cells (Figs. 29-5 & 29-6)
   - Goblet cells (Figs. 29-5 & 29-32).
   - Paneth cells (Figs. 29-12 & 29-14). The unusually large secretory granules of these cells contain lysozyme and α-defensins among other mediators. These granules would be highly eosinophilic by LM. Paneth cells are characteristically located in groups near the bottom of the intestinal glands of the small intestine.
   - Enteroendocrine cells (Fig. 29-16 & #14 in Fig. 29-5). Don’t worry about distinguishing the different types of enteroendocrine cells from one another.

4. Identify the inner circular and outer longitudinal layers of the muscularis externa and the neurons of the myenteric plexus, which lies between the two layers (Fig. 29-41).
5. In the large intestine identify a teniae coli (Figs. 29-28 & 29-29).
6. At the ano-rectal junction (Figs. 29-37) find the internal anal sphincter (smooth muscle) and notice how it represents a thickening of the inner circular layer of the muscularis externa. Circularly arranged muscle is what you would expect to find in a sphincter. Identify the external anal sphincter. This is skeletal muscle and is voluntary; the internal sphincter is involuntary.

LABORATORY 16 CHECKLIST
LOWER GI TRACT

LIGHT MICROSCOPY

<table>
<thead>
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<th>Description</th>
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<tr>
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<td>duodenum vs. jejunum vs. ileum</td>
<td>teniae coli</td>
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<td>goblet cell</td>
<td>mucosa (including lamina propria)</td>
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<tr>
<td>intestinal absorptive cell</td>
<td>submucosa</td>
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<tr>
<td>villus</td>
<td>muscularis externa</td>
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<tr>
<td>intestinal gland (crypt of Lieberkühn)</td>
<td>serosa/adventitia</td>
</tr>
<tr>
<td>Brunner’s glands</td>
<td>myenteric vs. submucosal plexus</td>
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<tr>
<td>Paneth cell</td>
<td>appendix</td>
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<tr>
<td>location of epithelial stem cells in small vs. large intestine</td>
<td>recto-anal junction</td>
</tr>
<tr>
<td>Peyer’s patch</td>
<td>perianal apocrine glands</td>
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<td>M cell</td>
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ELECTRON MICROGRAPHS

<table>
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<th>Description</th>
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<tr>
<td>villus vs. microvillus</td>
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<td>enteroendocrine cell</td>
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<td>goblet cell</td>
<td>Paneth cell</td>
</tr>
</tbody>
</table>

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 16: LOWER GI TRACT

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. Epithelial turnover is quite rapid in the GI tract. This fact is clinically significant since the rapidly dividing epithelial stem cells are often damaged by the treatments used to kill rapidly dividing cancer cells. This can lead to major problems with nutrition and fluid balance in cancer patients. Where are the epithelial stem cells located in the following organs: esophagus, stomach, small intestine and large intestine?

2. Describe three structural modifications that increase the surface area in the small intestine. Why is this important?

3. Describe the process by which IgA is transported across the intestinal epithelium into the lumen.

4. How does the presence of antibody in the intestinal lumen protect the body against invasion by that antigen?

5. Antimicrobial activity in the GI tract depends on a number of other mediators in addition to secretory IgA. How does the Paneth cell contribute to mucosal immunity?

6. What role do M cells play in the mucosal immune system? Where are they found?

7. Colon and cardiac stomach both lack villi and contain many mucus-producing cells. How can you distinguish between them morphologically?

8. What morphological characteristics allow you to distinguish duodenum from ileum and jejunum?

9. At two of the epithelial junctions in the GI tract a minimally keratinized stratified squamous epithelium abruptly changes to simple columnar or vice versa. This occurs at the gastroesophageal junction and at the rectoanal junction. Given this similarity, how can you distinguish these two junctions from one another?

10. At the gastroduodenal junction, how can you distinguish the two organs from one another since both are lined by simple columnar epithelium?
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 artfactually 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 cell 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

LIGHT MICROSCOPY

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<thead>
<tr>
<th>classical liver lobule</th>
<th>reticular fiber stroma</th>
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<tr>
<td>portal lobule</td>
<td>Kupffer cells</td>
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<tr>
<td>hepatic acinus (of Rappaport)</td>
<td>gall bladder</td>
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<tr>
<td>central vein</td>
<td>mucosa &amp; muscularis of gall bladder</td>
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<td>serosa/adventitia of gall bladder</td>
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<tr>
<td>portal canal</td>
<td>bile duct</td>
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<td>pancreas vs. parotid</td>
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<td>hepatocyte</td>
<td>pancreatic acinar cells</td>
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<tr>
<td>sinusoid</td>
<td>centroacinar cells</td>
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<tr>
<td>lipofuscin</td>
<td>islets of Langerhans</td>
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ELECTRON MICROGRAPHS

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<th>3 components of a portal triad</th>
<th>hepatocyte</th>
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<tr>
<td>portal vein vs. central vein</td>
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<td>absorptive cell of gall bladder epithelium</td>
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<td>Space of Disse</td>
<td>pancreatic acinar cell</td>
</tr>
<tr>
<td>endothelial cell</td>
<td>centroacinar cell</td>
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<tr>
<td>Kupffer cell</td>
<td>islet of Langerhans</td>
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</tbody>
</table>

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?
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 uninnferous 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

### LIGHT MICROSCOPY

<table>
<thead>
<tr>
<th>Structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>cortex of kidney</td>
<td>renal pyramids</td>
</tr>
<tr>
<td>medulla of kidney</td>
<td>renal papillas</td>
</tr>
<tr>
<td>renal corpuscle</td>
<td>renal columns (of Bertin)</td>
</tr>
<tr>
<td>glomerular (Bowman’s) capsule</td>
<td>area cribrosa</td>
</tr>
<tr>
<td>urinary space</td>
<td>minor calyx</td>
</tr>
<tr>
<td>podocyte</td>
<td>major calyx</td>
</tr>
<tr>
<td>vascular pole of renal corpuscle</td>
<td>interlobar artery</td>
</tr>
<tr>
<td>macula densa</td>
<td>arcuate artery</td>
</tr>
<tr>
<td>juxtaglomerular cells</td>
<td>interlobular artery</td>
</tr>
<tr>
<td>afferent/efferent glomerular arterioles</td>
<td>medullary ray</td>
</tr>
<tr>
<td>glomerular capillaries</td>
<td>cortical labyrinth</td>
</tr>
<tr>
<td>urinary pole of renal corpuscle</td>
<td>peritubular capillary</td>
</tr>
<tr>
<td>proximal convoluted tubule</td>
<td>vasa recta</td>
</tr>
<tr>
<td>thin limb of the loop of Henle</td>
<td>renal sinus vs. hilus of kidney</td>
</tr>
<tr>
<td>distal convoluted tubule</td>
<td>ureter</td>
</tr>
<tr>
<td>collecting tubule/duct</td>
<td>mucosa, muscularis &amp; adventitia/serosa of ureter &amp; urinary bladder</td>
</tr>
<tr>
<td>duct of Bellini</td>
<td>upper vs. lower end of ureter</td>
</tr>
<tr>
<td>renal lobe vs. renal lobule</td>
<td>urinary bladder (serosa vs. adventitia)</td>
</tr>
<tr>
<td>juxtamedullary nephron</td>
<td>penile urethra</td>
</tr>
<tr>
<td>cortical nephron</td>
<td></td>
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</tbody>
</table>

### ELECTRON MICROGRAPHS

<table>
<thead>
<tr>
<th>Structure</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>podocyte</td>
<td>filtration slit membrane</td>
</tr>
<tr>
<td>glomerular capillaries</td>
<td>urinary pole of renal corpuscle</td>
</tr>
<tr>
<td>parietal layer of Bowman’s capsule</td>
<td>proximal convoluted tubule</td>
</tr>
<tr>
<td>urinary space</td>
<td>thin limb of loop of Henle</td>
</tr>
<tr>
<td>vascular pole of renal corpuscle</td>
<td>distal convoluted tubule</td>
</tr>
<tr>
<td>macula densa</td>
<td>collecting tubule/duct</td>
</tr>
<tr>
<td>minimum filtration barrier</td>
<td>Transitional epithelium</td>
</tr>
</tbody>
</table>

**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?
LABORATORY 19

TESTIS & MALE REPRODUCTIVE TRACT

OBJECTIVES:

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

1. describe the general histology of the testis

2. describe the processes of spermatogenesis and spermiogenesis, and identify the various cells types present in seminiferous tubules

3. identify the Leydig cells of the testis, name the hormone they secrete, and describe the endocrine regulation of their activity

4. distinguish between the various parts of the male excurrent duct system: tubuli recti (straight tubules), rete testis, ductuli efferentes (efferent ductules), epididymis, and vas deferens (ductus deferens)

5. distinguish between the seminal vesicles and prostate by LM

6. describe the histology of the penis; distinguish between the corpora cavernosa and corpus spongiosum

LABORATORY:

Please study the following slides in your set:

I. TESTIS

Slide 80 (HU Box): Testis, or Slide 80: Testis

The testis is surrounded by a dense connective tissue layer, the tunica albuginea. Connective tissue septa radiate out from an area called the mediastinum (which may not be included on all versions of these slides) and divide the organ into lobules each containing 1-4 seminiferous tubules. The seminiferous tubules are highly coiled and are lined by an epithelium consisting of various types of spermatogenic cells and the Sertoli cells. The spermatogenic cells mature to become spermatozoa, while the Sertoli cells do not. In the seminiferous epithelium the most immature cells of the spermatogenic line are the spermatogonia. They rest directly on the basement membrane of the seminiferous tubule, while more mature cells are at higher levels in the epithelium, and the mature spermatozoa are by definition free in the lumen.

Identify a spermatogonium (it is not necessary to distinguish between type A and type B). Spermatogonia have either a round nucleus with a prominent nucleolus or an oval nucleus with less prominent nucleoli. Both types rest directly on the basement membrane of the seminiferous tubule.

Primary spermatocytes spend a long time (several weeks) in prophase of the first meiotic division, so you will see many of them in the tubules. While in prophase they have a round nucleus with an intact nuclear membrane and some evidence of
chromosome condensation. They are the largest cell type in the spermatogenic line. The first meiotic division produces two cells that are called secondary spermatocytes. These rapidly undergo the second meiotic division to give rise to spermatids. The second meiotic division is so rapid that secondary spermatocytes are rarely seen and you will not be asked to identify them.

Spermatids do not divide any further. Rather they undergo a process of morphological maturation called spermiogenesis, which results in the production of spermatozoa. Early spermatids are small round cells with round nuclei. They are located closer to the lumen than the spermatocytes. As spermiogenesis proceeds, the spermatids become elongated cells with elongated, heterochromatic nuclei. These late spermatids are oriented with their nuclear end (or head) embedded in the seminiferous epithelium and their cytoplasmic end (or tail) extending into the lumen. The last step in spermiogenesis is the loss of excess cytoplasm as a residual body. The rest of the cell is released into the lumen of the seminiferous tubule as a morphologically (but not physiologically) mature spermatozoan.

The spermatogenic cells are supported and nourished by Sertoli cells, which also phagocytize the residual bodies. Sertoli cells are the only cells that extend all the way from the basement membrane to the lumen although in a section is it usually impossible to tell that they do. Sertoli cells can be identified by their nucleus, which is characteristically irregular in shape (often with one deep invagination) rather than round, or oval. The nucleus is euchromatic and contains one or more large nucleoli.

Between the seminiferous tubules is the interstitial connective tissue, which contains Leydig cells (interstitial cells). These cells, which are responsible for the synthesis of testosterone, can occur either in groups or singly. The cells are large, rounded or polygonal, with a pale-staining vacuolated cytoplasm with lipid droplets that have been extracted during fixation.

The seminiferous tubules empty into short, narrow tubuli recti (straight tubules). The first part of the tubuli recti is lined almost exclusively by Sertoli cells, without any spermatogenic cells in the epithelium. This portion of the tubuli recti is short and not likely to be found on every slide. The remaining part of a tubulus rectus is a narrower, straight tubule that is lined by a conventional simple cuboidal epithelium. The tubuli recti empty into the rete testis, which is a network of irregular anastomosing channels (not straight tubules) lined by a low cuboidal epithelium. The channels of the rete testis are located in the mediastinum. If your section does not pass through the mediastinum, it will not demonstrate the rete testis.

**Slide 81 (HU Box): Spermatogenesis: Rat Testis, Iron Hematoxylin**

This excellent section of seminiferous tubules is stained with iron hematoxylin, which is particularly good for demonstrating nuclear morphology. Attempt to identify the same cell types and features described for slide 80, especially in the seminiferous tubules.

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II. MALE REPRODUCTIVE TRACT

A. EXCURRENT DUCTS

As a limbering up exercise, list (in order) the types of tubules that make up the excurrent duct system of the male reproductive tract. Then study the following slides:
Slide 24 (HU Box): Epididymis, Human

This slide contains sections through both the ductuli efferentes (efferent ductules) and the head of the epididymis. Both are lined by a pseudostratified columnar epithelium. You will be able to distinguish the efferent ductules because their lumen has a wavy edge due to the presence of groups of tall ciliated columnar cells alternating with groups of nonciliated cuboidal cells. Efferent ductules are the first tubule type to have a layer of true smooth muscle in their walls, although it is often quite thin and difficult to see.

The epididymis is a long convoluted tube lined by pseudostratified columnar epithelium. In contrast to the efferent ductules, the epithelium of the epididymis is of uniform height, and hence the lumenal surface is even. At the apical surface of the epithelial cells are stereocilia, which are long, branching microvilli rather than cilia. Here in the head of the epididymis the wall also includes a thin layer of circular smooth muscle. The number of muscle layers increases until there are three layers in the tail of the epididymis (inner longitudinal, middle circular, outer longitudinal). The number of muscle layers can thus be used to distinguish the head of the epididymis from the tail. The efferent ductules and the epididymis both resorb fluid produced by the Sertoli cells, thus creating a directional flow that helps to move the nonmotile sperm out of the testis and into the duct system. The epididymal epithelium also secretes products that modify the surface of the sperm and play a role in their maturation.

Slide 25 (HU Box): Vas Deferens, Human, c.s.

Slide 81: Vas Deferens Trichrome

Slide 81A: Spermatic Cord

The vas deferens (ductus deferens) is lined by a pseudostratified columnar epithelium with stereocilia, similar to that of the epididymis, except that the stereocilia tend to be shorter. The muscularis of the vas is much thicker than that of the tail of the epididymis. If the muscle has contracted during fixation, the wall will have longitudinal folds. These are temporary folds in contrast, for example, to the permanent folds of the mucosa in the oviduct. As in the tail of the epididymis, the muscle in the vas has inner and outer longitudinal layers and a middle circular layer. The extreme thickness of the muscularis relative to the size of the lumen is a key feature in distinguishing the vas from other muscular tubes such as oviduct or ureter. At high mag you could distinguish between these three tubules based on the type of epithelium that lines the lumen. What epithelium would you find in the oviduct? (Answer: Simple columnar with ciliated cells and peg cells) In the ureter? (Answer: Transitional)

The vas deferens is one component of the spermatic cord. On Slide 81A identify the other components including branches of the testicular artery and the pampiniform plexus of veins. Try to find the discontinuous strands of the cremaster muscle (skeletal muscle).

Slide 80: Testis

These slides contain sections through the epididymis, and in some cases through the rete testis and vas deferens (ductus deferens) as well. A few slides also include tubuli recti (straight tubules).

Slide 22 (HU Box): Penis, Human, c.s., or
Slide 85: Penis
This transverse section of the penis will demonstrate the arrangement of the three cavernous bodies: the paired corpora cavernosa, and the single corpus spongiosum surrounding the penile urethra. Notice that the erectile tissue of all three cavernous bodies consists of a network of thin-walled blood vessels called cavernous spaces, which are lined by endothelium and separated from one another by trabeculae of fibroelastic connective. Small arteries called helicine arteries are located in the trabeculae. They branch and empty into the cavernous spaces. During erection the blood flow through the helicine arteries into the cavernous spaces increases greatly. The two corpora cavernosa are both surrounded by a thick coat of connective tissue, the tunica albuginea. The tunica albuginea of the corpus spongiosum is a much thinner, more elastic layer. In slide 22, find an ischiocavernosus muscle partially surrounding the tunica albuginea of each corpus cavernosum, and the bulbospongiosus muscle partially surrounding the tunica albuginea of the corpus spongiosum. These skeletal muscles are present in the root of the penis (within the superficial pouch of the urogenital triangle) and in the proximal portion of the shaft, but are absent more distally. Compare this slide with slide 85, which shows a section of the distal shaft where these skeletal muscles are absent.

The penile urethra within the corpus spongiosum is lined mainly by pseudostratified columnar epithelium with patches of stratified columnar epithelium. Near the external orifice, it changes to stratified squamous epithelium. Mucus-producing glands of Littre secrete into the urethra. They are best seen on Slide 85.

B. ACCESSORY GLANDS

Slide 26 (HU Box): Seminal Vesicle, Human, c.s., or Slide 82: Seminal Vesicle

The seminal vesicles are paired exocrine glands, each formed by a single coiled tubule. Their duct joins the ductus deferens on each side of the body to form the paired ejaculatory ducts, which empty into the prostatic urethra. The most characteristic feature of a seminal vesicle is its complex pattern of interconnected mucosal folds, forming arches (arcades) that give a honeycomb appearance to the edge of the lumen at low magnification. The lumen of the gland is also fairly wide, and the central portion of the lumen is open. This open central portion of the lumen distinguishes the seminal vesicle from the ampulla of oviduct where the mucosal folds extend across the entire lumen. In seminal vesicle relatively little smooth muscle extends into the mucosal folds. Compare this with the prostate where the tissue between prostatic glands contains significantly more smooth muscle. The seminal vesicle can also be confused with the gall bladder, which often exhibits mucosal folding, although to a lesser degree than the seminal vesicle. The epithelium can sometimes help to distinguish between the two organs. Gall bladder has a simple columnar epithelium consisting of absorptive cells. Seminal vesicle is lined by a pseudostratified columnar epithelium, although the basal cells are so few in number that it may resemble a simple epithelium.

The secretions of the seminal vesicle are released into the ejaculatory duct by contraction of its smooth muscle during ejaculation. Is the smooth muscle of the seminal vesicle stimulated to contract by the sympathetic or the parasympathetic nervous system? (Answer: Seminal vesicle smooth muscle is stimulated to contract by the sympathetic nervous system. Remember the general rule that ejaculation is under sympathetic control while erection is parasympathetic. Seminal vesicle secretions are part of the ejaculate.) Name some of the secretory products of the seminal vesicle. (Answer: The seminal vesicle produces much of the fluid volume of the semen, and also secretes fructose, fibrinogen, vitamin C and prostaglandins.)
Slide 83 and 83B: Prostate & Seminal Vesicles

Although labeled “Prostate and Seminal Vesicles”, Slide 83 actually shows the prostate and ejaculatory ducts. The morphology of the ejaculatory ducts is very similar to that of the seminal vesicles. To locate the ejaculatory ducts, hold your slide up against a light-colored background and look for the two large structures located near the center of the section. The ejaculatory duct is formed by the union of the duct of the seminal vesicle and the vas deferens. It enters the prostate and empties into the posterior wall of the prostatic urethra (not present on most versions of this slide). Therefore we can tell these are the ejaculatory ducts and not the seminal vesicle because they are within the prostate (surrounded by prostatic tissue) and because there are only 2 of them (one from each seminal vesicle). Sections this large that passed through the seminal vesicles would show many cross sections through the coiled tubule that forms that organ.

The prostate is composed of many branched tubuloalveolar glands whose ducts open separately into the prostatic urethra. Unless your specimen shows evidence of benign prostatic hypertrophy (BPH), the diameter of each individual prostatic gland is usually quite small compared to the diameter of an ejaculatory duct. The stroma of the prostate contains much more smooth muscle than does the seminal vesicle. It is in the form of thin strands of smooth muscle cells blending in with the connective tissue between the glands. Concretions are also more characteristic of the prostate than of the seminal vesicle.

The glands of the prostate are of three types: mucosal glands (located just beneath the epithelium of the urethra), submucosal glands (located somewhat further from the urethral lumen) and prostatic glands proper or main prostatic glands (most peripherally located). You should understand that it is normally the hypertrophy of mucosal glands and/or the fibromuscular stroma that causes benign prostatic hypertrophy, which is common in males over the age of 50. In contrast, the submucosal glands and the prostatic glands proper are more often the sites of prostatic carcinomas.

Slide 84 and 84A: Prostate

The prostate surrounds the neck of the bladder and makes up the wall of part of the urethra (prostatic urethra). Slide 84A and some versions of Slide 84 also include the prostatic urethra. What types of epithelium line the three portions of the male urethra (prostatic, membranous and penile)? (Answer: There is transitional epithelium in the first part of the prostatic urethra. This makes sense because it is continuous with the bladder, which is also lined by transitional epithelium. Pseudostratified columnar epithelium with patches of stratified columnar lines most of the rest of the urethra, except that it changes to stratified squamous near the distal end of the penile urethra. This also makes sense since this is continuous with the stratified squamous epithelium on the skin of the penis.)

Slide 83 (HU Box): Prostate, Older Human

In this specimen from an older male, observe the enlargement (hypertrophy) of some of the prostatic glands.
III. ELECTRON MICROSCOPY (RHODIN)

A. TESTIS

1. Study the organization of the cell types present in a seminiferous tubule (Fig. 33-3). Locate spermatoogonia (it is not necessary to distinguish between type A and type B), primary spermatocytes and spermatids. Be aware that Sertoli cells are the only cell type to extend from the basal lamina to the lumen. In this non-human specimen, the nuclei of Sertoli cells are at the basal end of the cell, near the basal lamina. In human seminiferous tubules the Sertoli cell nuclei are closer to the lumen (i.e., near the center of the cell rather than at its basal end).

2. Examine the Sertoli cells in Fig. 33-10 and observe their characteristic irregular euchromatic nuclei. There are usually multiple prominent nucleoli and one deep cleft in the nucleus, although these features may not always be included in the plane of section. Sertoli cells are connected to one another by tight junctions that divide the tubule into an adlumenal and an ablumenal (basal) compartment. What cell types are found in each compartment? (Answer: The basal compartment contains spermatoogonia and early primary spermatocytes. The adlumenal compartment contains later primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa.)

3. Be able to identify a Leydig cell. Fig. 33-4 shows the typical location of these cells between seminiferous tubules, but the cells in this micrograph are unusual in that they lack clear evidence of cytoplasmic lipid droplets. Leydig cells produce a steroid hormone (testosterone) and usually have the typical appearance of steroid secreting cells: numerous lipid droplets, abundant SER, and mitochondria with tubular cristae (see Fig. 33-5). Leydig cells also contain rod-shaped cytoplasmic crystals called crystals of Reinke and numerous lysosomes, neither of which is evident in these micrographs.

4. Study the stages of spermiogenesis.

   In the **Golgi phase** (Figs. 33-14 & 33-15) the Golgi complex becomes more elaborate and the acrosomal granule and acrosomal vesicle form. The centrioles migrate to the opposite pole of the nucleus.

   In the **cap phase** the acrosomal vesicle and granule spread to cover the anterior half of the nucleus (and are then known as the acrosome) (Figs. 33-16). One of the centrioles begins to form the axoneme, which makes up the core of the tail of the mature spermatozoan.

   In the **acrosome phase** the spermatid elongates and orients itself with its head buried in the Sertoli cell cytoplasm and its tail extending into the lumen. Microtubules form a temporary cylindrical structure called the manchette (Fig. 33-17) that extends from the posterior edge of the acrosome into the developing tail. The centrioles become modified to form the connecting piece or neck of the mature sperm. Nine outer dense fibers (Fig. 33-19) develop from the centrioles and lie external to the axoneme. Finally the manchette disappears and mitochondria wrap in a tight helix around the ring of dense tubules to form the middle piece of the tail (Fig. 33-19).

   There are no micrographs of the maturation stage. During this stage the residual cytoplasm of the spermatid is shed and phagocytized by Sertoli cells. The resulting mature spermatozoan is thus released into the lumen.
5. Observe the following parts of a mature spermatozoan in cross & longitudinal section.

   Head (Figs. 33-22 & 33-23)
   Tail which is divided into the:
   Neck (#8 in Fig. 33-23) - the region containing the modified centriole.
   Middle piece (Figs. 33-19 & 33-23) - defined by the mitochondrial sheath.
   Principal piece (Figs. 33-20, 33-24 & 33-25) - defined by a fibrous sheath that has replaced the mitochondrial sheath. In the principal piece the outer dense fibers gradually disappear.
   End piece (Fig. 33-21) - defined as the region distal to the fibrous sheath. It contains only the axoneme.

B. MALE REPRODUCTIVE TRACT

1. Observe the thin layer of smooth muscle surrounding the duct in the epididymis (Fig. 33-32). This increases in thickness as you approach the tail of the epididymis, but never becomes as thick as the muscular layer seen in the vas deferens. Identify the stereocilia (Fig. 33-33). Note that they are not cilia at all, but are actually long microvilli that have an absorptive function.

2. Study the vas deferens (ductus deferens). Notice that its epithelial cells also have stereocilia (Fig. 33-37), although they are usually somewhat shorter than those of the epididymis. By LM, observe the thickness of the muscular coat in the vas deferens and its arrangement in three layers (Fig. 33-35).

3. Compare LM of the seminal vesicle (Fig. 33-39 & 33-40) and prostate (Fig. 33-44 & 33-45). The tubules of the seminal vesicle have a wider lumen with a characteristic honeycomb effect created around the edge by extensive mucosal folds (Fig. 33-39 vs. 33-44). Compare the seminal vesicle with the ampulla of the oviduct (Fig. 34-24), which also has extensive folds. Note that in the ampulla the mucosal folds tend to fill the entire lumen rather than being restricted to the edge of the lumen.

4. Study the penis by LM in Fig. 33-48. Identify the two corpora cavernosa and distinguish them from the unpaired corpus spongiosum. The corpus spongiosum surrounds the penile urethra. The tunica albuginea surrounding the corpus spongiosum is much thinner than that which surrounds the corpora cavernosa. Compare this cross-section of the penis with the section of the prostate in Fig. 33-44.

   The cavernous spaces in the penile erectile tissue (Fig. 33-50) are thin-walled blood vessels. They are lined by endothelium, and separated from one another by trabeculae containing fibroblasts, collagen fibers, elastic fibers, smooth muscle cells and helicine arteries. (Fig. 33-49 & 33-50). The helicine arteries empty into the cavernous spaces.
## LABORATORY 19 CHECKLIST
### TESTIS & MALE REPRODUCTIVE TRACT

**LIGHT MICROSCOPY**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>testis</td>
<td>spermatic cord</td>
</tr>
<tr>
<td>tunica albuginea</td>
<td>pampiniform plexus</td>
</tr>
<tr>
<td>mediastinum</td>
<td>testicular artery in spermatic cord</td>
</tr>
<tr>
<td>seminiferous tubule</td>
<td>cremaster muscle</td>
</tr>
<tr>
<td>spermatogonium</td>
<td>penis</td>
</tr>
<tr>
<td>primary spermatocyte</td>
<td>corpora cavernosa</td>
</tr>
<tr>
<td>early spermatid</td>
<td>corpus spongiosum</td>
</tr>
<tr>
<td>late spermatid</td>
<td>cavernous spaces of erectile tissue</td>
</tr>
<tr>
<td>spermatozoan</td>
<td>trabeculae of erectile tissue</td>
</tr>
<tr>
<td>Sertoli cell</td>
<td>helicine arteries</td>
</tr>
<tr>
<td>Leydig cell</td>
<td>penile urethra</td>
</tr>
<tr>
<td>tubulus rectus</td>
<td>glands of Littre</td>
</tr>
<tr>
<td>rete testis</td>
<td>seminal vesicle</td>
</tr>
<tr>
<td>efferent ductule</td>
<td>prostate</td>
</tr>
<tr>
<td>epididymis</td>
<td>prostatic concretions</td>
</tr>
<tr>
<td>vas deferens (ductus deferens)</td>
<td></td>
</tr>
</tbody>
</table>

**ELECTRON MICROGRAPHS**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>seminiferous tubule</td>
<td>Sertoli cell</td>
</tr>
<tr>
<td>spermatogonium</td>
<td>Leydig cell</td>
</tr>
<tr>
<td>spermatocyte</td>
<td>acrosome</td>
</tr>
<tr>
<td>early spermatid</td>
<td>spermatozoan</td>
</tr>
<tr>
<td>late spermatid</td>
<td>neck, middle piece, principal piece &amp; end piece of sperm</td>
</tr>
</tbody>
</table>

**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 19: TESTIS & MALE REPRODUCTIVE TRACT

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. What is meant by the terms “spermatogenesis”, “spermiogenesis” and “spermiation”?
2. Are spermatogonia arrested in meiosis just as primordial oocytes are in the ovary?
3. What creates and maintains the separation between the basal and adlumenal compartments of the seminiferous tubules?
4. What cell types are found in each of the compartments (basal and adlumenal) in a seminiferous tubule? How do developing cells move from one compartment to the other?
5. Why is it necessary to maintain two separate compartments?
6. What is a “stage” of the seminiferous epithelium?
7. How are the stages arranged in a human seminiferous tubule?
8. What is a “cycle” of the seminiferous epithelium?
9. How would you distinguish between the head, neck, middle piece, principal piece, and end piece of a spermatozoan by electron microscopy?
10. Describe how the acrosome forms during spermiogenesis. What does it contain and what is its function?
11. How does the morphology of a Leydig cell reflect its function? Consider its appearance by LM and EM.
12. What mechanism assures that there will be a high concentration of testosterone in a seminiferous tubule?
13. What is the pampiniform plexus? Why is it important in sperm production?
14. What are stereocilia? Where are they found in the male reproductive tract and what is their function?
15. What is the functional significance of the fact that the tunica albuginea is much thinner around the corpus spongiosum of the penis than around the corpora cavernosa?
LABORATORY 20
OVARY & FEMALE REPRODUCTIVE TRACT

OBJECTIVES:

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

1. identify the parts of a normal ovary: hilus, medulla, cortex, germinal epithelium, and tunica albuginea

2. identify ovarian follicles of all stages: primordial follicle, unilaminar primary follicle, multilaminar primary follicle, secondary follicle, mature (Graafian) follicle, and atretic follicle

3. identify the following structures within ovarian follicles of the appropriate stage: membrana granulosa cells, cumulus oophorus, corona radiata, theca interna, theca externa, zona pellucida, antrum, oocyte (and its cytoplasm vs. nucleus vs. nucleolus)

4. identify a corpus luteum and its component parts: the theca lutein and granulosa lutein

5. identify a corpus albicans

6. list the hormones produced by a follicle and a corpus luteum; know what cell type produces each, the target organ and action of each, and how the secretion of each hormone is regulated

7. describe the general histology of the oviduct and compare and contrast its different parts: infundibulum, ampulla, isthmus & interstitial (intramural) segment

8. identify the myometrium, endometrium, stratum basale, stratum functionale, endometrial glands and spiral arteries of the uterus

9. identify the following stages of the uterus based on changes in the endometrial glands and the stroma: proliferative stage, early secretory stage (~ day 17), late secretory stage, & menstrual stage

10. correlate the morphology of the uterus with that of the ovary at different stages of the menstrual cycle

11. describe the general histology of the cervix and vagina. Know which cells are studied in the Papanicolaou method (Pap smear).

LABORATORY:

Please study the following slides in your set:

I. OVARY
   Slide 85 (HU Box): Ovary, or
   Slide 86: Ovary
   In these sections of the ovary, the follicles are in various stages of development. Identify examples of primordial follicles, unilaminar primary follicles and multilaminar primary follicles. Primordial follicles lie just deep to the tunica albuginea and consist of the oocyte surrounded by a single layer of flat follicular cells. When the follicle is
stimulated, the follicle cells become cuboidal to form unilaminar primary follicles. Once they become cuboidal, the follicle cells are known as granulosa cells or membrana granulosa cells. They divide to form multiple layers, at which point the follicle is a multilaminar primary follicle. Next find either a secondary follicle or a mature (Graafian) follicle. Both have a central, fluid-filled cavity called the antrum, but a Graafian follicle is much larger than a secondary follicle. It should fill the entire width of the cortex and even cause a bulge on the surface of the ovary. Identify the following features in a secondary or Graafian follicle:

- oocyte
- zona pellucida
- membrana granulosa
- theca interna
- theca externa
- antrum
- cumulus oophorus
- cells that will become the corona radiata after ovulation

What hormones do the membrana granulosa cells secrete? (Answer: Mainly estrogens, especially estradiol) What hormones do the theca interna cells secrete? (Answer: They produce androstenedione, which serves as a precursor for estrogen production in the membrana granulosa cells.)

Slide 27 (HU Box): Ovary, Corpus Luteum of Ovulation, Human, or Slide 87 and 88: Ovary

Slides 27HU & 87 contain a large corpus luteum that formed from a ruptured follicle following ovulation. The corpus luteum includes a folded mass of granulosa lutein cells derived from the membrana granulosa cells of the follicle, and theca lutein cells derived from the theca interna. The theca lutein cells are small and dark-staining. They form thin strands of cells located between the folds of the granulosa lutein cells. The granulosa lutein cells make up the bulk of the corpus luteum. They are large cells whose cytoplasm is rich in lipid droplets. What hormone does each cell type secrete? (Answer: Theca lutein cells produce androgens such as androstenedione. Granulosa lutein cells convert the androgen to estradiol, and also produce progesterone from cholesterol supplied mainly by LDLs. The LDLs are carried to the granulosa lutein in blood vessels that grow into that layer following ovulation. Recall that the membrana granulosa of the follicle was avascular, and hence the cells had limited access to LDLs at that stage.) Find some of the small blood vessels that are present within the granulosa lutein.

In most of these slides the lumen of the corpus luteum still contains clotted blood, so the structure should more properly be called a corpus hemorrhagicum rather than a corpus luteum. Slide 88 shows several older corpora lutea where the folds of granulosa lutein cells have become so extensive that a central lumen is no longer visible.

The cells of the corpus luteum eventually degenerate, and are phagocytized by macrophages. All that remains of the former corpus luteum is a scar of poorly vascularized dense connective tissue called a corpus albicans. This structure persists through several cycles before it becomes indistinguishable from the surrounding stroma. Next to the corpus luteum on some versions of slides 27HU and 87 is a corpus albicans.
Slide 86 (HU Box): Ovary, Corpus Luteum of Ovulation, C.T. Stain

Identify the corpus luteum and confirm this one does not have a theca lutea. This is because it is from an animal species where the theca lutein cells intermingle with the granulosa lutein so that there is no separate theca lutein layer. Human corpora lutea have a distinct theca lutein. Note the lipid-rich cytoplasm of the granulosa lutein cells and the vascularity of the corpus luteum. Locate the dense connective tissue layer called the tunica albuginea near the surface of the ovary, just deep to the germinal epithelium. In places, the germinal epithelium may have been lost during tissue preparation.

Look at any of the ovary slides to try to identify an atretic follicle. Atretic follicles are those that are in the process of degenerating, and will never be ovulated. Atresia can occur at any stage of follicular development, and therefore atretic follicles differ greatly in size and morphology. During the atresia of any follicle, the oocyte dies and the granulosa cells detach from their basal lamina and degenerate. Often all that remains of a primary follicle that became atretic is a collapsed zona pellucida. In an atretic secondary follicle you may see individual granulosa cells or clumps of cells that have been sloughed off into the antrum. Sometimes the basal lamina of the membrana granulosa becomes thickened to form a glassy membrane.

II. FEMALE REPRODUCTIVE TRACT

Slide 23 (HU Box): Fallopian (Uterine) Tube, Fimbriated End, Human, c.s.

The lumen of the entire oviduct is lined by a simple columnar epithelium containing ciliated cells and non-ciliated (peg) cells. Peg cells are secretory in nature. The apical end of the peg cells often extends further into the lumen of the oviduct than do the ciliated cells, making them stand out like little pegs.

The oviduct can be divided histologically into four segments: the funnel-shaped free end (the infundibulum), the ampulla, the isthmus and the interstitial (intramural) segment, which passes through the wall of the uterus. As you progress from infundibulum to interstitial segment, the folding of the mucosa becomes less pronounced, the thickness of the smooth muscle layer gradually increases, and the diameter of the lumen changes.

Slide 23 is a transverse section through the uterine tube at the level of the infundibulum. Notice that the mucosal folds extending into the lumen are large and complex. Identify the peg cells and ciliated cells, noting the excellent preservation of cilia in these slides. The lumen of the infundibulum is in direct continuity with the peritoneal cavity, and the tube ends in numerous fringe-like projections called fimbriae. The same epithelium that lines the lumen extends over the fimbriae and then becomes continuous with the simple squamous epithelium that covers the outer surface of the rest of the uterine tube. Look around the outer edge of the section for areas where the section passed through fimbriae that were curving backward from the mouth of the uterine tube. The presence of fimbriae conclusively identifies this as the infundibulum. Another less obvious clue is the numerous large, thin-walled veins in the fimbriae and in the wall of the tube. Engorgement of these vessels helps extend the fimbriae toward the ovary just prior to ovulation, so that they almost surround the ovary, helping to ensure that the ovulated oocyte will enter the uterine tube rather than becoming lost in the peritoneal cavity. Note the presence of numerous nucleated cells in the lumen of the oviduct. What are these cells and what does their presence suggest? (Answer: They are neutrophils, and their presence suggests a mild case of acute inflammation, i.e., salpingitis).
Slide 88 (HU Box): Fallopian (Uterine) Tube, Ampulla, Human, c.s., or Slide 89A (Several Versions): Uterine Tube, Ampulla

Identify the three layers that make up the wall of the uterine tube: the mucosa, the muscularis and the serosa. The characteristic features of the ampulla that distinguish it from the other regions of the uterine tube are: a mucosa that is thrown into elaborate highly branched folds, fewer large veins in the serosa as compared to the infundibulum, the absence of fimbriae, and a wide lumen.

Slide 89 (HU Box): Fallopian (Uterine) Tube, Isthmus, Human, c.s., or Slide 90: Uterine Tube (Oviduct)

Compare the isthmus of the oviduct to the infundibulum and ampulla. In the isthmus the lumen is narrower, the mucosal folds are greatly reduced in number and height and the extent to which they branch, and the muscularis becomes thicker.

Slide 28 (HU Box): Uterus, Follicular Phase (Proliferative), Human Slide 92: Uterus Early Proliferative, and Slide 92B: Uterus, Proliferative

Identify the endometrium and the myometrium. The outermost layer of the uterus is not present on most of these slides. It is primarily a connective tissue layer containing large blood vessels, and is called the perimetrium. Most of the outer surface of the uterus is covered by a mesothelium called the peritoneum. The presence of a mesothelium makes that portion of the perimetrium a serosa. Over the more inferior portions of the uterus, near the cervix, there is no mesothelium, so the outermost layer of the perimetrium is an adventitia in that location.

See if it is possible to distinguish the two layers of the endometrium, stratum basale and stratum functionale, on your slide. Generally the stroma of the stratum basale is more cellular, and the presence of many nuclei makes this area stain somewhat darker than the stratum functionale. The stratum functionale is shed during menstruation while the stratum basale is not. During the proliferative phase of each uterine cycle, under the influence of estrogen, the stratum functionale is restored.

The lumen of the uterus is lined by a simple columnar epithelium containing ciliated cells and secretory cells, although it is not always easy to distinguish between them. This surface epithelium invaginates to form glands that contain mainly secretory cells. The glands are present in both the stratum functionale and the stratum basale. The portion of the glands in the stratum basale is coiled and does not undergo major changes in morphology during the uterine cycle. In contrast, the morphology of the glands in the stratum functionale undergoes marked changes, so that their appearance depends on the stage of the menstrual cycle.

In Slide 28HU & Slide 92 the uterus is in the proliferative phase of the cycle, and the glands in the functionale are long, narrow, relatively straight tubules. There is considerable mitotic activity in the epithelial cells of the glands and in stromal cells. The spiral arteries are short (having lost their terminal portions during menstruation) and only moderately coiled. They are therefore fairly inconspicuous.

Slide 92B could be considered to be in either the late proliferative or early secretory stage, in that the glands are becoming slightly wavy rather than straight.

Observe the myometrium and note that the smooth muscle cells are organized into bundles, and that neighboring bundles are oriented in many different directions rather than forming distinct layers.
In the secretory phase, the myometrium is under the influence of progesterone. The epithelium of the glands begins to secrete a mucoid material and there are major changes in glandular morphology, especially in the stratum functionale. These include the following:

- The **glands** first take on a coiled or corkscrew appearance where the entire gland curves back and forth like a sine wave. The walls of the gland are parallel to one another. Later in the secretory phase the glands become sacculated and the epithelial cells form tooth-like projections that give a serrated appearance to the edge of the lumen (the sawtooth appearance).

- In the early secretory phase (~ day 17) the **nuclei** of the gland cells are displaced toward the apical end of the cell by the accumulation of glycogen in the basal cytoplasm. A few days later the glycogen moves toward the apical cytoplasm to be secreted, and the nuclei return to their basal position.

- The **spiral arteries** elongate toward the surface of the endometrium, become progressively more coiled, and develop thicker walls, making them more apparent in the mucosal stroma. (These vessels, although called arteries, usually have the morphology of arterioles in most of the stratum functionale.) To find these vessels, look for multiple cross sections through an arteriole clustered together within a small area of the stroma. The multiple sections are probably all part of a single coiled vessel; in favorable specimens the sections through the vessel will be arranged in a column oriented from the lower portion of the stratum functionalis toward the surface epithelium.

- **Edema** of the stroma becomes even more pronounced, and then near the end of the secretory phase decreases precipitously. The excess extracellular fluid is removed by the venous system, causing irregularly shaped, thin-walled venous lacunae to become evident. The entire thickness of the functionalis decreases, which may contribute to the sacculation of the glands.

- The **stromal cells** near the lumen of the uterus take on a "predecidual" appearance, meaning that they appear more like epithelial cells than fibroblasts, i.e., the cells and nuclei are rounder and surrounded by more cytoplasm. Later these cells will also cluster around spiral arteries, and finally become widespread throughout the stroma. (One version of Slide 93 labeled “Uterus Secretory H&E” is at a later stage than the other, and the predecidual changes are more pronounced.)

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**Slide 91: Uterus Menstrual**

The menstrual phase of the uterine cycle is characterized by:

- Reduced **edema** of the stroma.
- Infiltration of the stroma by **leukocytes**, including neutrophils & eosinophils (leukocyte infiltration actually begins in late secretory phase).
- A **surface epithelium** that is disrupted or missing.
- **Erythrocytes** free in the stroma due to bleeding from ruptured spiral arteries.
Menstruation occurs as a result of ischemia caused by prolonged contractions of the spiral arteries. This leads to necrosis of the terminal portions of the arteries themselves as well as the surrounding tissue. When the spiral arteries then re-open, clumps of necrotic stratum functionale are washed away by blood flowing out of the disrupted arteries. This combination of necrotic tissue and blood constitutes the menstrual flow. Notice that the stratum basale is not affected because it is supplied by straight arteries that do not undergo prolonged contractions. Be aware that both spiral and straight arteries arise from the radial arteries of the uterus. The blood supply of the uterus is arranged as follows: the uterine artery gives off 6-10 arcuate arteries in the myometrium, radial arteries branch from these and enter the endometrium, straight arteries branch from the radial arteries in the stratum basale & supply it, and the main trunk of the radial artery continues on into the stratum functionale as a spiral artery.

The leukocytic infiltration typical of the menstrual phase serves a protective function. Normally the epithelial lining of an organ is its first line of defense, helping to keep antigens (including micro-organisms) from penetrating into the wall of the organ. Since the epithelium is lost during menstruation, an alternate defense mechanism, namely leukocytes, must be employed.

As the proliferative phase of the next cycle begins, epithelial cells from the bases of the glands in the stratum basale will begin to divide and migrate up to restore the glands in the stratum functionale as well as the surface epithelium. Stromal cells will divide and begin to produce the extracellular matrix of the growing stratum functionale, and the cells in the walls of the spiral arteries will divide as the vessels grow toward the uterine surface.

Slide 31 (HU Box): Cervix Uteri, Human, l.s., or
Slide 93A (Several Versions): Uterus L.S. Cervix, or Cervical Canal

The cervix is the inferior portion of the uterus, but it has a distinctly different histology and physiology from the rest of the uterus. The cervix is continuous with the uterine cavity superiorly through a constriction called the internal os and with the vagina inferiorly through the external os. The cervical canal or endocervix is the lumen of the cervix; it runs from internal os to external os. The mucosa of the cervical canal is lined mainly with tall mucus-secreting columnar cells that also line the highly branched, tubular cervical glands. The glands are oriented obliquely with reference to the cervical canal. The necks of the glands may become occluded, resulting in the accumulation of mucus within their lumens and forming large cysts called Nabothian cysts (Nabothian follicles). Is the surface layer of the cervix shed during menstruation? [Answer: No, the cervix is considered part of the uterus by gross anatomists, but it differs significantly from the rest of the uterus in terms of its histology and physiology. One example is that the rest of the uterus sheds its surface layer (stratum functionalis) whereas the cervix does not.]

Compare the connective tissue underlying the cervical epithelium with that of the rest of the uterus. You should notice that the cervix contains relatively dense collagenous connective tissue.

The lower part of the cervix extends downward into the lumen of the vagina. Clinicians refer to this part as the portio vaginalis. Histologists sometimes reserve this term to refer to the point of transition between the simple columnar epithelium of the cervix and the stratified squamous epithelium of the vagina. The exact location of this squamo-columnar junction changes with age.

Examine the epithelium of the vagina on these and the following slides.
Slide 90 (HU Box): Vagina, Human
Slide 94B: Vagina, Human, and
Slide 94: Urethra & Vagina, Mallory Trichrome

The epithelium lining the vagina is minimally keratinized stratified squamous. (Note that some versions of Slide 90 HU are somewhat atypical in that the epithelium looks like stratified cuboidal in many places.) Glycogen is a major component of the vaginal epithelial cells, and the pale-staining regions in the cytoplasm of the surface cells are areas where extensive glycogen deposits were extracted during fixation. The glycogen accumulates during the proliferative phase under the influence of estrogen, and is shed into the lumen in increasing amounts during the secretory phase under the influence of progesterone.

In addition to the high glycogen content, other characteristics of vagina are:

- The connective tissue layer beneath the epithelium usually has long papillae that project into the overlying epithelium.
- A rich complex of veins is present in the connective tissue layer (especially on Slide 90 HU), and these become engorged with blood during sexual excitement.
- The vagina is generally devoid of glands. Vaginal epithelium is lubricated in part by mucus from cervical glands, and in part by fluid transudate from vessels in the vaginal wall.

External to the connective tissue layer is a muscularis composed of smooth muscle and finally an adventitia.

In Slide 94, be sure to distinguish the vagina (at the edge of the section) from the cross section of the urethra near the center. Both structures are lined by minimally keratinized stratified squamous epithelium, although other types of epithelia (including transitional) line the more proximal portions of the urethra. Both the vagina and the urethra open into the vestibule (not shown on this slide), which is the space between the two labia minora.

III. ELECTRON MICROSCOPY (RHODIN)

A. OVARY
1. In Figure 34-3, identify the germinal epithelium on the surface of the ovary. It was at first thought that oocytes developed from these cells, but in fact they do not. Identify tunica albuginea, primordial follicles, the large follicle labeled secondary follicle, zona pellucida, membrana granulosa cells (#7), basal lamina, theca interna (note the lipid droplets beginning to accumulate in the cytoplasm of the cells as they differentiate into steroid-producing cells) and theca externa. The identification of the large follicle as a secondary follicle could be argued, since there is no obvious antrum visible in this micrograph. However the figure legend says this section came from a follicle such as that seen in Fig. 34-2, where an antrum is clearly visible.

2. Compare the relatively squamous follicular cells of a primordial follicle to the cuboidal follicular cells of a unilaminar primary follicle (Fig. 34-4 vs. 34-5). (NOTE THAT DESPITE WHAT THE LEGEND TO FIG. 34-4 CLAIMS, PRIMORDIAL FOLLICLES CONTAIN PRIMARY OOCYTES, NOT PRIMORDIAL GERM CELLS. PRIMORDIAL GERM CELLS ARE MITOTIC CELLS THAT EXIST ONLY DURING
FETAL DEVELOPMENT. PRIMARY OOCYTES ARE ARRESTED IN THE FIRST MEIOTIC PROPHASE.) Primary oocytes persist until a few hours before ovulation, at which time they complete the first meiotic division (giving off the first polar body), enter the second meiotic division, and become arrested as secondary oocytes. The second meiotic division is not completed until the time of fertilization.

3. Observe the structure of a secondary follicle (Figs. 34-6 & 34-7). (NOTE THAT SECONDARY FOLLICLES ALSO CONTAIN PRIMARY OOCYTES, NOT SECONDARY OOCYTES AS THE LEGEND TO FIG. 34-6 CLAIMS.) Note the beginning of antrum formation as fluid has accumulated in the extracellular spaces between granulosa cells. These spaces will enlarge and fuse to become the antrum. Identify the nucleolus, nucleus and cytoplasm of the oocyte, the zona pellucida, and the cells that will form the corona radiata upon ovulation. The cortical granules in the superficial cytoplasm of the oocyte (Fig. 34-7) are modified lysosomes. Their contents will be released at fertilization (as part of the cortical reaction) and will then alter the properties of the zona pellucida (zona reaction). Notice that cytoplasmic processes from both the oocyte and the corona radiata cells penetrate into the zona pellucida (Fig. 34-10), where they contact one another and form gap junctions.

4. Identify the granulosa cells, theca interna (identified by the lipid droplets in some cells) and theca externa of a secondary follicle at high magnification (Fig. 34-12). Note that a basal lamina separates the granulosa cells (membrana granulosa) from the theca interna. This basal lamina can thicken greatly during atresia to form the glassy membrane of atretic follicles. Be sure you can distinguish the basal lamina from the zona pellucida (Fig. 34-6).

5. Observe how the granulosa lutein cells of the corpus luteum have acquired the ultrastructural appearance of steroid-secreting cells (Fig. 34-21). Compare this with the relatively undifferentiated appearance these cells had when they were membrana granulosa cells in a follicle (Fig. 34-6).

6. Study the light microscopic appearance of a corpus albicans (Fig. 34-19). A corpus albicans is the remnant of an old corpus luteum; it is composed of connective tissue. At a higher magnification than shown here, you would see that it contains relatively few cells (mostly fibroblasts) that are scattered among abundant collagen fibers. Compare this with the corpus luteum, which is highly cellular and contains little connective tissue of any kind.

7. Fig. 34-20 illustrates an atretic follicle with a thickened, folded glassy membrane (#2), and a remnant of the collapsed zona pellucida (#4). These features are not easy to see in this micrograph. Both would stain eosinophilic with H&E. Consult the videodisk for better examples of atretic follicles.

B. FEMALE REPRODUCTIVE TRACT

1. In the oviduct note the simple columnar epithelium with its ciliated cells and secretory (peg) cells (Fig. 34-27). Peg cells may have microvilli, but no cilia (note the absence of basal bodies in their apical cytoplasm).

2. Compare the LM appearance of uterine glands in the proliferative stage (Figs. 34-29) with the secretory stage (Figs. 34-32). Fig. 34-32 actually shows glands with
coiled or corkscrew glands rather than sacculated, so it might better be labeled early or mid-secretory, instead of late. Observe that in the secretory stage glycogen appears to be released from the cell in an apocrine fashion (Figs. 34-34 & 34-35).

3. Study the cervical glands (Figs. 34-40 to 34-43). Notice that, like the surface epithelium of the cervix, the epithelium of the glands consists mostly of mucous cells. There is also an occasional ciliated cell.

4. Be aware that the vagina is lined by a nonkeratinized (minimally keratinized) stratified squamous epithelium (Fig. 34-46), since the surface cells still contain recognizable nuclei. Note the presence of glycogen in the vaginal epithelium (Figs. 34-48 & 34-49).
LABORATORY 20 CHECKLIST
OVARY & FEMALE REPRODUCTIVE TRACT

LIGHT MICROSCOPY

<table>
<thead>
<tr>
<th>OVARY</th>
<th>OVIDUCT</th>
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<tbody>
<tr>
<td>tunica albuginea</td>
<td>ciliated vs. peg cells</td>
</tr>
<tr>
<td>germinal epithelium</td>
<td>fimbriae</td>
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<tr>
<td>cortex vs. medulla of ovary</td>
<td>infundibulum vs. ampulla vs. isthmus</td>
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<tr>
<td>primordial vs. primary vs. secondary follicle</td>
<td>UTERUS</td>
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<tr>
<td>oocyte</td>
<td>endometrium</td>
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<tr>
<td>zona pellucida</td>
<td>myometrium</td>
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<tr>
<td>follicular cell</td>
<td>uterine glands</td>
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<tr>
<td>membrana granulosa</td>
<td>stratum basale</td>
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<tr>
<td>theca interna vs. theca externa</td>
<td>stratum functionale</td>
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<tr>
<td>antrum</td>
<td>spiral artery</td>
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<tr>
<td>cumulus oophorus</td>
<td>proliferative vs. secretory vs. menstrual phase</td>
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<tr>
<td>corpus luteum</td>
<td>CERVIX</td>
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<tr>
<td>granulosa lutein cells</td>
<td>cervical glands</td>
</tr>
<tr>
<td>theca lutein cells</td>
<td>Nabothian cyst</td>
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<tr>
<td>corpus hemorrhagicum</td>
<td>squamo-columnar junction with vagina</td>
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<tr>
<td>corpus albicans</td>
<td>VAGINA</td>
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<tr>
<td>atretic follicle</td>
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<tr>
<td>glassy membrane</td>
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ELECTRON MICROGRAPHS

| primordial vs. primary vs. secondary follicle| membrane granulosa cells                     |
| oocyte                                      | theca interna vs. theca externa               |
| zona pellucida                              | antrum                                        |
| follicular cells                            | ciliated vs. peg cells of oviduct             |

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 20: OVARY & FEMALE REPRODUCTIVE TRACT

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. Are the oocytes in primordial follicles interphase cells or are they arrested at some stage in mitosis or meiosis?

2. How does the location of a follicle in the ovary change as it develops?

3. Distinguish between the structure of primordial follicles, unilaminar primary follicles, multilaminar primary follicles and secondary follicles. When does the zona pellucida appear? The antrum?

4. How would you distinguish between a secondary ovarian follicle and a Graafian follicle? How can you use the oocyte to help you make this distinction?

5. Theca interna cells of the developing follicle produce androgens, but when the same cells become theca lutein cells in the corpus luteum they produce estrogen. What prevents them from producing estrogen during the follicular phase?

6. What is the only type of ovarian follicle that ever contains a secondary oocyte? Does it always contain a secondary oocyte?

7. The oocyte that is ovulated after the first meiotic division is a secondary oocyte. What is its DNA content and chromosome number? Why is the first meiotic division sometimes called a reduction division?

8. Why does the infundibulum of the oviduct have many large, thin-walled veins in its wall?

9. Why is the lumen of the ampulla of the oviduct almost completely filled by mucosal folds?

10. Compare the muscularis externa of the oviduct with that of the ureter and small intestine in terms of the number of muscle layers and their orientation.

11. How would you distinguish between parts of these different tubes (oviduct, ureter and small intestine) that have the same muscle arrangement?

12. Name the three phases of the uterine cycle. With which phases of ovarian function do these phases coincide?

13. What causes the stratum functionalis of the uterus to be shed during menstruation, whereas the stratum basalis is retained?

14. Why is it important that the stratum basalis is not lost during menstruation?

15. Distinguish between the uterus, the cervix and the vagina in terms of their epithelium and glands.

16. In what ways do the properties of cervical mucus change during the menstrual cycle? Why are these changes beneficial?
17. What is meant by the “fundus” of an organ? Name some organs that have a fundus.

18. The vagina does not undergo the dramatic cyclic changes that the uterus does, but there are some variations in the structure and function of the vaginal epithelium during the menstrual cycle. Describe these.

19. In a Pap (Papanicolaou) smear, also called a cervical smear, epithelial cells are swabbed from the region of the squamo-columnar junction, spread on a glass slide, fixed, stained and examined for evidence of a variety of conditions including cervical carcinoma. What cell type is the pathologist most interested in, and what morphological changes correlate with possible malignancy?

Congratulations!
You have completed Microscopic Anatomy! We hope you have enjoyed the course, and we wish you continued success and satisfaction in all your remaining studies.
In addition to the policies outlined for IFM students earlier in the lab manual, the following items apply specifically to IMS students.

**Quiz Remediation**
A quiz that is missed due to medical school interviews cannot be made up.

**Scavenger Hunt Remediation**
Scavenger hunts that have been missed can be made up, providing that the student has contacted the IMS Laboratory Course Director (Judy Rae Churchill, Ph.D.) prior to the date of the event and made appropriate arrangements. The reason for treating scavenger hunts differently from quizzes is that scavenger hunts are a valuable learning opportunity as well as an evaluative tool, and can easily be modified to provide individualized scavenger hunts for use in remediation.

**Semester Letter Grades**
90% or better is a guaranteed A.
A* and B+ are given to IMS students whose grades fall into the range in which IFM students would receive a grade of Highly Satisfactory. It generally includes a very small percentage of the class.
B is considered an average grade that will include the class mean. This grade normally accounts for the largest percentage of the class.
Averages that fall into the range where IFM students would receive a Marginal Unsatisfactory will be given a D in the IMS program.
Averages that fall into the range where IFM students would receive an Unsatisfactory will be given an F in the IMS program.

**First and Second Semester Remediation Exams**
Remediation exams are offered in the first and second semester, but an individual student may remediate only once during the course. Thus, students who remediate their first semester grade would be ineligible for remediation at the end of the second semester. Remediation is offered only to students with a semester average of C- or D. Each exam has approximately 100 written questions. There is no “practical” component, although the written questions can include drawings and diagrams. Students who score 70% or better on a remediation exam will have their semester grade changed to a C. The grade cannot be changed to anything higher than a C regardless of the score on the exam.

**Why Students Cannot Remediate a Grade of F**
IMS students with a grade of F can't remediate because we treat IMS students in the same way that we deal with IFM students. IFM students who receive a Marginal Unsatisfactory (equivalent to a D) in Microscopic Anatomy can remediate by examination, but those who receive an Unsatisfactory (equivalent to an F) cannot. They are required to retake the course or take an approved summer school course. This is a policy of the medical school that applies to all the larger courses.
Final Exam
IMS students are not required to take the cumulative final exam in Microscopic Anatomy. The reason for this is that the final exam includes material from the first semester, and IMS students have already been graded on their first semester work.
When comparing the scores of the IMS students to IFM students to assign second semester letter grade cut-offs for IMS, we will use the IFM scores from the end of the Abdominal Pain module (i.e., their scores on the final exam will not be included).
Any IMS student who wants to take the exam as a diagnostic test (i.e., just to see for their own satisfaction how they would do) may do so, but it will not count toward their second semester grade.

The Final Exam is the Drexel Exemption Exam
The final exam of the IMS course also serves as the exemption exam for the IFM course at Drexel University College of Medicine (DUCOM). Therefore, any IMS student who hopes to attend DUCOM in the IFM program, and who wishes to exempt from the Microscopic Anatomy course must have taken and passed the final exam at the end of their IMS course. No additional exemption exams will be given during the summer or at the beginning of the following academic year.
Students get one chance to pass the final exam for exemption purposes. If they fail the exam at the end of their IMS course and still wish to be enrolled in the IFM curriculum rather than PIL, they must take the IFM Microanatomy course over again at DUCOM. Exemption is good for 5 years.