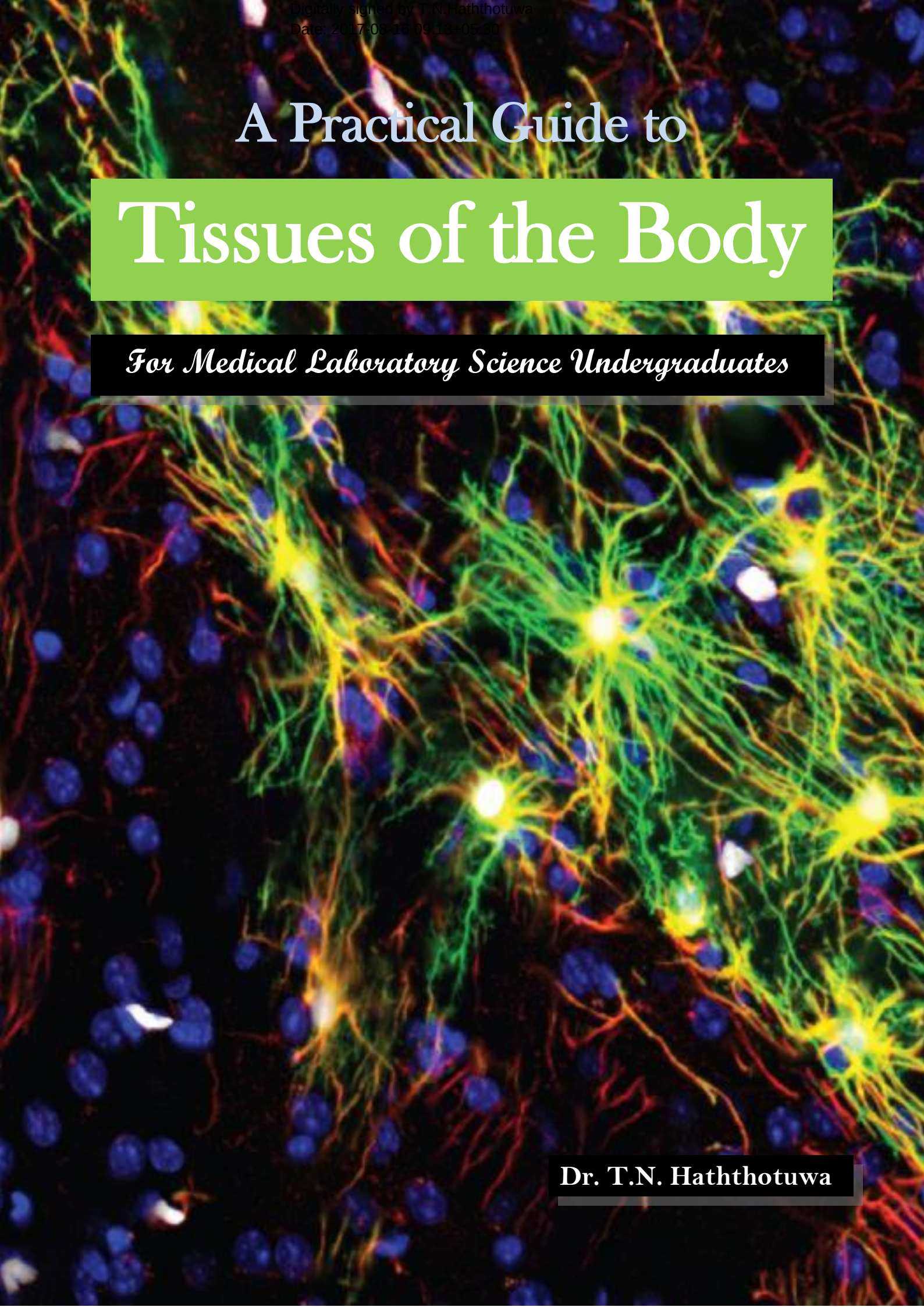
A Practical Guide to

Tissues of the Body

For Medical Laboratory Science Undergraduates



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For Medical Laboratory Science Undergraduates

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PREFACE

Initially practical sessions for Tissues of the Body were conducted using a limited number of demonstration slides focused under high power objective without allowing students to move the slide or study the whole section individually. This method seriously limited the knowledge to be gained through the lab practicals. Tissue sections vary greatly depending on the site of specimen collection, how sections are cut and stained, as well as the skill of the technician. Therefore, it is a must to allow students to study the whole tissue section by themselves at preliminary stage and allowing them to inquire about unidentified structures in tissue sections.

This manual has been prepared as a guide to focus the class slides and to encourage students to study tissue sections by themselves. Though, due to the limited availability of certain slides some slides are still being focused under high power objective without allowing students to move the slide. Anyhow, many slides from different tissue sections are being preparing nowadays to provide slide for each student and to let them study individually in future.

This manual describes only the class slides which will be available during each practical class. With the addition of new slides this practical manual will be updated by the author in each year. Knowledge of histology is ever changing with new researches and their findings. Therefore, it is your responsibility to go through the newest edition of text books and upgrade your knowledge.

Dr. T. N. Haththotuwa

August 2017

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REVIEWERS' NOTE

It has been my pleasure to review this practical laboratory guide for the introductory course in Histology "Tissues of the Body," expertly prepared by Dr. T. N. Haththotuwa attached to the Department of Basic Sciences, Faculty of Allied Health Sciences, University of Peradeniya. For the student of histology, there is no substitute for the ability to study properly stained microscopes slides freely, moving throughout all regions of each tissue and organ, examining structures of interest at higher magnification and being able to recognize such structures at various magnifications. However, such an initial exploration of the body's microscopic structure, like any venture into new territory, is best accomplished with a guidebook or a map to explain what one is seeing and indicate the points of major interest and importance. Dr. Haththotuwa's laboratory guide is designed to provide students with exactly those directions and that information.

Students using this practical guide together with a standard textbook of histology, such as that of Junqueira or Wheater's, will find it much easier to gain the basic knowledge needed to understand the microscopic structure of our bodies' organs, without which the physiological function of the organs is much more difficult to comprehend. Throughout the histology course students should continuously correlate information gained from the textbook and lecture presentations with an appreciation of the cells and other structures to which their attention is guided in the laboratory sessions. In addition to such correlations, students will also be helped by preparing written summaries of key facts, by discussing among themselves how a tissue's structure facilitates its basic function, and by making sketches of various important microscopic structures.

As you begin the study of the body's tissues, I congratulate you for the opportunity to learn this most interesting and intimate aspect of human anatomy and I encourage you to make the most of this opportunity by taking full advantage of this Practical Manual and your instructor's expertise.

Dr. Anthony L. Mescher, Ph.D.
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Tissues of the body course overview

(ML1203)

Tissues of the body subject is a one credit course which includes approximately 15 hours of lectures and 30 hours of practicals. Under this course you will be studying the microscopic structure of normal cells and tissues of the human body along with their functional adaptations which facilitate its function.

Histological structure of a tissue has a great relationship with its function and it determines the functions of different organs and tissues. Therefore, knowledge of histology is aid in guessing the function of an unknown tissue by its structure. In addition, sound knowledge of normal structure is prerequisite to study the histopathology, in which you will be studying the microscopic structure of an abnormal/diseased tissue with functional abnormalities. The light microscope and the stained thin sections of tissues are used as the major tools for studying histology.

There are four basic tissues of the body; connective, epithelial, nerve and muscle tissues. These four tissues are organized into form various organs of the body. Under this course you will be studying the four basic tissues first and histology of different organ systems subsequently.

At the successful completion of the course, the students should be able to:

- identify cells and tissues of the body under the light microscope.
- describe characteristic histological features of different tissues.
- list the functions of different tissues of the human body.
- briefly explain the procedure of tissue processing and slide preparation.
- identify different special stains used in histological sections and state their uses.

Recommended textbooks

1. Haththotuwa, T. N. Tissues of the Body Practical Guide: For Medical Laboratory Science Students (2017).
2. Young, B., Heath, J. W., Stevens, A., Lowe, J. S., Wheater, P. R., & Burkitt, H. G. Wheater's Functional Histology_A Text and Colour Atlas.
3. Mescher, A. L. Junqueiras's Basic Histology Text and Atlas (2013).

Basic regulations of the practical class

- Eating, drinking and smoking are prohibited in the laboratory area.
- Wearing laboratory coats is a must.
- Mobile phones are prohibited to use inside the laboratory.
- Handle the microscopes and slides with care.
- Read the instructions displayed beside the microscope carefully.
- Practical manual and one of the recommended text books should read and prepare for the practical prior to the practical class.
- Available histology text books should bring to the practical class.
- Work as a group and share your knowledge with others.
- Use a science book with plain pages to enter the practical.
- Each practical entering should be start with mentioning the
    - Practical number and title
    - Date
- Microscopic appearance of cells and tissues under low and high power objectives should be drawn clearly and coloured.
- Finish your write up individually. In case of coping you will be getting zero marks.
- Questions given in the handout should be answered during the practical class and answer sheets will be collected at the end of each class.
- Practical books should be submitted to the office two days after the practical class to obtain a grade.
- 80% attendance for the practicals and lectures compulsory to face the end examination.
- Any breakage/damage to the class slides should be immediately informed to the lecturer in charge and you are required to pay the fine to the department/faculty.
- All the slides must be returned and microscopes should be cleaned and covered after each and every practical class.
- Contact lecturer in change /one of the instructors for any clarifications.

At the end of each and every practical class following steps must be followed to wind up,

- Turn the nosepiece and take lowest power lens into position.
- Use coarse adjustment knob to lower the stage far down as much as possible.
- Remove the slide from the stage.
- Clean the lenses and the stage if necessary.
- Move the stage to backwards/ towards the arm.
- Move the slide bar/holder towards the left.
- Be sure that the iris diaphragm is completely open by turning the level to the left.
- If the condenser is movable use the condenser arm to elevate it all the way up.
- Reduce the light intensity and turn of the microscope.
- Unplug the microscope.
- Wrap the cord around the plug and fix it to the backside of the arm.
- Cover the microscope with dust cover when it is not in use.
- Carry the microscope with both hands. Grasp the arm with one hand and place the other hand under the base for support.
- All the slides should be cleaned with lens paper or tissue to remove fingerprints, oil or dirt. If the slide cannot be cleaned with a dry tissue, use alcohol.

Practical 01: Introduction to tissues of the body

All the tissues and organs of the body are composed of cells and the extracellular matrix. **Tissues** are defined as group of differentiated cells which are highly specialized to perform specific function. Therefore microscopic structure of a tissue always related to its function.

E.g. pancreatic acini produce digestive enzymes. All the enzymes are proteins. Thus these cells are having lot of RER and ribosome within their cytoplasm.

Histology is the study of both cells and the matrix collectively whereas **cytology** is the study of cells only. **Light microscopes (LM)** with 0.2 μm optical resolution are used for this purpose. It permits the observation of large area of a specimen and identification of nucleus, nucleoli and other tissue features. The image under LM is formed when light passes through the tissue. Hence the tissue must be cut into very thin slices (1- 10 μm thickness). It produces colour images due to staining in which different dyes are added in order to increase visualization and contrast. Though, the maximum available magnification is limited to ×1000 in most LMs. Thus, it provides less detail on individual cell structure.

Electron microscopes (EM) with superior resolution (20 nm) and magnification (×100 000) are frequently used to study cell structure and organelles. Instead of light beam, electron beam is passing through the specimen. Therefore, it requires ultra thin specimens (50-100 nm). However, it produces black and white images and this method is comparatively costly and time consuming.

Objectives

At the end of this practical student should be able to,

- identify the differences between light micrographs and electron micrographs.
- list the cell organelles that can be seen under LM and EM.
- focus tissue slides under LM and examine under 4×, 10×, 40× and oil immersion.
- identify different cutting sections (transverse/longitudinal/oblique).
- identify cells and cell organelles under LM.
- describe the cell/tissue structure.
- recognize stages of mitosis.

Task 1

Observe the given light micrographs and electron micrographs.

1) List pros and cons associated with electron microscopy.
2) State the differences between light and electron micrographs.
3) List the cell organelles that can be seen under LM and EM.
4) Identify the pointed cell structures in electron micrographs.
5) Note the cell shape, shape of the nucleus, number of nuclei per cell, presence/absence of nucleoli and colour of cytoplasm in provided light micrographs.

Task 2

Observe the given slides with your naked eye. Note the number of tissue sections on the slide (one/more), nature of the specimen (thick/hollow), size and shape of the section, edges of the section (sharp/not) etc.

6) Read the slide label and write the information available on it.
7) What are the advantages of observing a slide with your naked eye?

Task 3

First observe the colour card beside the microscope. Then go through the focused slides under different magnifications. Draw the structures that you observe under each magnification and colour them. Label the structures properly. Note the following features while observing,

- **Cell shapes** (squamous, columnar, cuboidal, polygonal, wedge shape, irregular)
- **Cell size** (bigger/small)
- **Cytoplasm** (colour-pale pink/dark purple, margins-clear/not, nucleus: cytoplasm ratio, uniform staining/not)
- **Nucleus**

  Shape (circular/elongated)

  Number of nuclei/ cell

  Staining (E.g. dark purple, pale pink)

  Location of nucleus (Basally, centrally, eccentrically located)

  Presence/absence of nucleolus

Record the slide name and magnification with each drawing.

Task 4

Focus the provided slide under light microscope following each step described in page number 04 and wind up steps described in page number 05.

8) State the advantage of focusing a slide under low power objective first rather than focusing directly under 40× objective/oil immersion.

Task 5

Observe the focused slides under microscope "A", "B" and "C". Recognize different cutting sections in each. (Transverse, longitudinal, oblique)

A:----------

B:----------

C:----------

Task 6

Identify the artefact in each focused slide.

(Air bubbles, tissue folds, dye particles, knife marks)

A:----------

B:----------

C:----------

D:----------

Task 7

Identify the mitotic figures in focused slide.

(Prophase, Metaphase, Anaphase, Telophase)

Practical 02: Slide preparation for light microscopy

This demonstration practical is intended to introduce histotechnology techniques to first year undergraduates and to make them aware how LM slides that they are going to examine throughout this course is prepared. Small pieces of tissues with few millimeter thicknesses need to be obtained during autopsy or surgery in order to prepare these histology slides.

Objectives

At the end of this practical student should be able to,

- list the steps involve in histology slide preparation.
- state the importance of major processing steps.
- identify the apparatus used in slide preparation and state their uses.
- recognize commonly used stains and their uses.
- identify artifacts in the tissue slides.

Go through the stations that demonstrate the steps and apparatus used in slide preparation. Record the major steps. Draw and label the apparatus and mention their uses.

Station 1

Examine the provided biopsy samples and tissue sections. Note the size of each tissue section. Then compare the fresh biopsy sample with formalin fixed sample provided next to it. Also note the fixative to tissue volume ratio in the container.

Q1) State the differences between fresh sample and fixed sample.

Q2) List the advantages of fixing the tissue.

Station 2-Dehydration

Dehydration is the first step of the process. The tissue specimen is transferred into a series of alcohol solutions of increasing concentration until, water-free or absolute alcohol container is reached.

Q3) What is the purpose of this step?

Station 3-Clearing

Next the tissue section is dipped in xylene solution to replace alcohol in the specimen with xylene. Because wax going to be used in next step is largely immiscible with alcohol.

Station 4-Embedding

Observe the different embedding molds and plastic cassettes provided at this station. Tissue specimen is kept at the middle of the mold and liquid paraffin is poured onto it. Then the mold top is covered with plastic cassettes and wax is allowed to solidify. Examine prepared paraffin blocks.

Q4) What is the advantage of wax embedding?

Station 5-Sectioning

Hardened paraffin blocks are cut into thin slices using an instrument call rotary microtome. Therefore, many slides can be prepared with one paraffin embedded block. Each slice of paraffin contains a thin section/s of tissue. Observe the demonstration that shows you how to use the rotary microtome and resulting thin sections which are colourless and transparent.

Station 6-Mounting on a slide

Thereafter, thin sections are transferred to a tissue floatation bath containing warm water and floated. These sections are next mounted on slides avoiding formation of wrinkles. Finally slides are dried using an incubator.

Staining tissue sections with Haematoxylin and Eosin

Most tissues are colourless and transparent when thinly sectioned. It must be stained with a coloured dye in order to study the structural detail. Different dyes in stains are responsible for the colours seen under LM in tissue slides but not the true colours of that particular tissue. Haematoxylin and Eosin combination is the most commonly used stain in histotechnology. Haematoxylin is a basic dye which stains acidic cell structures like nuclei, ribosomes and RER in purple/bluish in colour. Whereas, eosin is an acidic dye which stains basic cell components like cytoplasmic proteins in pink/ pinkish red in colour. The staining procedure will be discussed in detail under histotechnology subject.

Task: Observe the colour of provided stains in jars and draw them.

- Haematoxylin
- Eosin
- Methylene blue
- Leishman stain
- Toluidine blue

Practical 03: Connective Tissues

Introduction

Connective tissues are the most abundant and widely distributed tissue type in the body. It has many specific functions such as, binding and support, protection, energy storage, thermal insulation and transportation of substances. Connective Tissues are made of three main components: matrix, fibers and cells. Each major type of connective tissue has its own fundamental cell type in both immature and mature forms. E.g. Cartilage → Chondroblast → Chondrocyte. Connective tissues are also home to many other cell types including mast cells and macrophages.

Further connective tissues can be rigid (bone), flexible (adipose), or fluid (blood). Some tissues are avascular (Cartilage), poorly vascularized (dense connective tissue) and some have rich blood supply (bone). Under this practical you will be looking at histological sections of major as well as some specialized connective tissues.

Objectives

At the end of this practical student should be able to,

- identify the different types of connective tissues under LM.
- list the characteristic histological features in each CT.
- recognize different cell and fiber types under LM (fibroblast, mast cells, elastic and collagen fibers).
- state one location of CT and its major function.
- identify and name specific stains.

1) Loose connective tissue/Areolar tissue- Spread preparation-Verhoeff stain

Areolar connective tissue is the most widely distributed loose connective tissue in the body. It serves as a packaging material between other tissues. Consist of fewer collagen and elastic fibers going in all directions. Identify the elastic fibers (dark brown-black). These fibres are thin, homogenous threads that branch are anastomosing to form a loose network. See also the collagenous fibres (pink). The majority of the cells seen here are fibroblasts. Present in papillary layer of the skin dermis, between mm, lamina propria of the intestine.

2) Dense connective tissue – Capsule of the Spleen – H & E stain

Identify the dense connective tissue in the capsule and trabeculae of the spleen. This tissue is composed of collagen fibers (pink) arranged in a regular manner.

3) Dense connective tissue –dermis of the skin – H & E stain

Identify the dense connective tissue found in the dermis of the skin. Note the irregularly arranged collagen fibers. These have a wavy appearance.

4) White adipose tissue- Hypodermis/around kidneys-H & E stain

Note the large lipid droplet occupying whole cell cytoplasm. Lipid has dissolved out during preparation leaving white vacuoles and thin rim of cytoplasm at the periphery (Chicken wire appearance). Nucleus pushed towards one side by large fat droplet (eccentric Nucleus).

5) Adipose tissue-Osmium stain-colour card no 1

Note fat lobules stained with black colour inside the adipocytes and surrounding nerve bundle.

6) Brown adipose tissue- H & E stain-colour card no 2

Adipocytes are arranged into lobules separated by fibrous septa. Note pink stained cells at the centre of lobules and pale staining cells at periphery.

Q1) Mention one reason for staining difference.

7) Brown adipose tissue - H & E stain-colour card no 3

Note foamy appearance/ multilocular nature of stored lipid in adipose tissue under high magnification. Nuclei are eccentrically located. Note the eosinophilic cytoplasm and rich network of capillaries.

8) Hyaline Cartilage- Trachea -H & E stain

Identify the chondrocytes (cells) lie in lacunae. Usually 2 to 4 cells cluster together. Note amorphous amount of matrix produced by chondroblast.

9) **Osseous tissue**-colour card no 4

Note central Haversian canal, osteocytes in lacuna and canaliculi. What is lamella?

Practical 04: Epithelial Tissue

Introduction

The epithelia can occur as a sheet of cells on the surface or they can down growth into the underlying connective tissue forming glands. Surface epithelia occur either covering body surfaces exposed to outer environment or lining the lumen of the cavities and the tubes. E.g. Epidermis of the skin and gut epithelium. All the epithelia are supported by a basement membrane which separates the epithelium from underlying supporting tissues. Blood vessels never penetrate into the epithelial cell layer thus depends on diffusion of oxygen and metabolites. Further epithelial cells are polarized, with one side facing the basement membrane (the basal surface) and the other facing outwards (the apical surface).

Epithelia may classify as **simple** or **stratified** depending on the number of cell layers. A simple epithelium has a single layer of cells in which all the cells are in contact with the basal lamina. Whereas, stratified epithelium has more than single cell layer and only basal layer of cells make contact with the basal lamina. Epithelia are further classified into **squamous**, **cuboidal** and **columnar** based on the cell shape. Similarly, stratified epithelia also classify based on the surface cell shape. Considering both classifications, there are six types of epithelia have recognized and there are two specialized epithelia call **pseudostratified epithelia** and **transitional epithelia.**

Objectives

At the end of this practical student should be able to,

- identify different types of epithelia under LM.
- list the characteristic features in each epithelia.
- recognize surface specializations in epithelia.
- state one location where different types of epithelial occur.

Examine the focused slides and colour cards and recognize the type of epithelia present. Note that actual shape of the cell and number of cell layers can be altered in sections due to extraneous factors such as cutting plane of the section, thickness of the section and shrinkage during fixation.

1) **Simple squamous epithelium-**Lung tissue- H & E

Do not move the slide. Examine the slide under 10 ×objective first and then under 40 ×. Note that alveoli lining with simple squamous epithelia. Single layer of flatten, irregular and elongated cells with elongated nuclei which usually bulge out into the surface. Cytoplasm is scanty. Examine the Bowman's capsule slide focused next to this microscope.

2) Simple cuboidal epithelium- Thyroid- H & E

Do not move the slide. Thyroid gland is made up of follicles filled with colloid (pink colour material at the middle). Follicles are lined by simple cuboidal epithelium. Epithelial cells appear square and the nucleus is usually rounded and located in the centre of the cell. If the follicle is full, the cuboidal epithelium may flatten and appear more or less similar to simple squamous. Examine the next focused slide and answer the questions.

i) List three structures that you could observe in this slide.
ii) List two types of epithelia present in this section?
iii) What is the surface specialization in pointed structure? Mention the importance of it.

3) Simple columnar epithelium- Gallbladder- H & E

Cells are taller and appear columnar in sections perpendicular to the basement membrane. The height of the cells may vary from low to tall columnar, depending on the site or degree of functional activity. The nuclei are elongated and may be located towards the base, the centre or occasionally the apex of the cytoplasm.

4) Simple columnar epithelium- Small Intestine (duodenum, jejunum, ileum) - H & E

Villi of the small intestine have simple columnar epithelial lining.

i) Identify the surface specialization at the pointer.
ii) Mention specific term used to describe it and its function.
iii) Examine the slide (**colon/large intestine**) focused under next microscope. What is the specific characteristic feature of the epithelium?

5) Simple columnar with cilia- Oviduct/Fallopian tube - H & E

Examine the focused slide and note the cilia of apical surface. Columnar cells appear stratified in certain areas.

i) What is the function of cilia in oviduct?

6) Pseudostratified columnar with stereocilia-Vas deferens & epididymis - H & E

Examine the focused slides and note the stereocilia at the tip of the pointer.

i) State the differences between cilia, stereocilia and microvilli.

ii) What is the function of stereocilia in epididymis and vas deferens?

7) Pseudostratified ciliated columnar with goblet cells- Trachea - H & E

Also call as respiratory epithelium. Compare the circular nuclei of the basal cells and elongated nuclei of the cells on top of it. Nuclei are dispersed at different levels (mainly centrally or basally). Note the cilia and goblet cells.

8) Stratified squamous epithelium- Esophagus and uterine cervix - H & E

Examine the shape of the basal cell layer (cuboidal) and note the change in shape of superficial cells (flattened). Cells lying at the middle are polygonal in shape. In these two locations it acts as lining epithelia.

9) Stratified squamous keratinized epithelium- Skin - H & E

Here the stratified keratinized epithelium of the skin acts as covering epithelium. Note the appearance of keratin granules and disappearance of nuclear material while cells are moving towards the surface. Thick keratin layer at the top most layers is made up of dead squamous cells filled with keratin protein.

10) Stratified cuboidal epithelium- Ducts of salivary gland - H & E

Examine thin epithelium with two/three layers of cuboidal cells.

11) Stratified columnar epithelium-Ducts of salivary gland - H & E

Epithelial height is comparatively higher. Note basal cuboidal and surface columnar cells.

12) Transitional epithelium-Urinary bladder and Ureter - H & E

Found only in lining of the urinary tract. Increased number of cell layers and bigger surface cells can be noticed in non-stretched stage. Basal cells are roughly cuboidal. Intermediate cells are polygonal. Surface cells, large and round with white spaces in cytoplasm (dome/umbrella shape). Some cells may contain 2 nuclei.

Practical 05: Muscle Tissue

Introduction

The major function of the muscle tissue is to generate a force by contractions. Sometimes contractile cell function as single-cell units:

- **Myoepithelial cells**
  Present around secretory glands, function is to expel secretions from glandular acini.
- **Myofibroblasts**
  Contractile role in addition to secretion of collagen
- **Pericytes**
  Smooth muscle-like cells that surround the blood vessels

Other forms of contractile cells function by forming multicellular contractile units termed muscles. Such muscle cells can be divided into three types: Skeletal, Cardiac and Smooth.

- **Skeletal muscle** is responsible for the movement of the skeleton as well as organs such as the globe of the eye and the tongue. The arrangement of the contractile proteins actin and myosin give rise to the appearance of prominent cross-striations
- **Smooth muscle:** contractile proteins do not give the histological appearance of cross-striations. Also called as visceral muscle.
- **Cardiac muscle:** Have many structural and functional characteristics intermediate between those of skeletal and smooth muscle. Major function is to continuous rhythmic contraction of the heart.

Muscle cells of all three types are surrounded by an **external lamina** which binds individual muscle cells into a single functional mass.

Objectives

At the end of this practical student should be able to,

- identify different types of muscle tissue (Transverse and cross sections).
- list the characteristic histological features of the each type.
- state the locations where different types of muscle tissue can be seen.

Examine the focused slides and colour cards and recognize the characteristic features of each muscle tissue type. Note the number, shape and location of the nuclei, cross striations, size shape and arrangement of the muscle fibers as well as intercalated disks.

1) Skeletal muscle- Tongue - H & E

These are cross sections of tongue. Do not move the slide. Examine the slide under 10 × objective first and then under 40 ×. Note direction and arrangement of fibers, cross striations, number of nuclei and their position. Draw fiber in both longitudinal and transverse sections.

2) Cardiac muscles- Heart - H & E

Do not move the slide. Identify cardiac muscle fibers, cross striations, intercalated discs and Purkinje fibres. Purkinje fibres are modified cardiac muscles specialized to conduct impulses. Locate directly under the endocardium. They are larger and pale staining compared to cardiac muscles.

i) Why Perkinje fibres are pale staining compared to cardiac muscles?

3) Smooth muscles- Small intestine - H & E

Examine the cross sections and draw the transverse and cross sections of smooth muscle in inner circular and outer longitudinal layers of SI.

4) Smooth muscles- Vas deference - H & E

Note thick muscularis propria with inner longitudinal, middle circular and outer longitudinal smooth muscle layers.

5) Skeletal or smooth muscles- Esophagus - H & E

Examine the focused slide inner circular and outer longitudinal layers of clearly distinguishable.

- Upper third of the tube: bundles of skeletal mm predominate
- Middle third: gradual transition from striated to smooth mm
- Lower oesophagus: entirely of smooth mm

i) How muscularis propria get arranged in the stomach?

Practical 06: Nervous Tissue

Introduction

Neurons are the basic functional units of nervous tissue. They are highly specialized to transmit nerve impulses. Two main parts of the nervous system:

- **Central Nervous System**: brain and spinal cord
- **Peripheral Nervous System**: nerves and ganglia

Nervous tissue is made up of **Neurons** and **Neuroglia** (glial/supportive cells). Astrocytes, oligodendrocytes, microglia and ependymal cells represent the supporting cells of the CNS whereas Schwann cells (neurolemmocytes) and satellite cells represent that of PNS. Neuron is consisting of nerve cell body and two types of cell processors: dendrites and axon.

- **Dendrites**: one or more, highly branched short tapering processes, receive the stimuli
- **Axon**: commonly referred as nerve fibres, single, conduct stimuli away from the cell body, cylindrical process up to 1 meter in length.

In addition, nerve cell body consist of large vesicular nucleus, prominent nucleolus and basophilic **Nissl bodies** in cytoplasm. Based on number of processors neurons are classified into **unipolar** (pseudounipolar), **bipolar** and **multipolar** neurons. Schwann cells and oligodendrocytes make up the myelin sheath around the axon in PNS and CNS respectively. Even though myelin sheath is remain unstained under H & E.

Macroscopically, CNS is divided into two areas called as **grey matter** and the **white matter**. In CNS nerve cell bodies are located in the grey matter. Accumulations of nerve cell bodies outside the CNS referred as **ganglia**. Collection of axons forms the peripheral nerves and white matter. CNS also made up of myelinated and non-myelinated axons.

Objectives

At the end of this practical student should be able to,

- identify the arrangement of nerve bundle and label epi-, peri- and endoneurium.
- identify glial/supportive cells in CNS and PNS.
- recognize the arrangement of grey matter and white matter in CNS.
- identify nerve cell bodies and its nuclei, nucleoli, Nissl bodies and processors.

1) **Spinal cord -** Transverse section-H & E

Focus the slide under low power and screen the slide. Examine the inner grey matter, central canal, white matter and pia matter at the periphery. The delicate layer of collagen fibres covering the nerve tissue called as pia mater. Then focus the grey matter under 40 × and observe the nerve cell bodies surrounded by the white colour spaces. These spaces are there due to the shrinkage of nerve cell bodies during preparation. Identify the following structures:

- Larger nucleus
- Prominent nucleoli
- Nissl bodies
- Dendrites

Rests of the smaller nuclei are belong to glial cells. Then examine the white matter consisting cross sections of cylindrical axons covered by unstained clear areas representing myelin.

2) Spinal cord- (special stain)

Examine the slide under 10 × and 40 ×. Note all the above described features in this slide. Focus the central canal under high power and observe the ependymal cells lining the central canal.

Q1) What is the function of ependymal cells in CNS?

3) Brain (class slide)- H & E

Move the slide and change the magnification. Note nerve cell bodies and nuclei of glial cells.

4) Optic nerve (special stain)

This slide shows a transverse section of nerve bundle. Recognize the epineurium, perineurium and endoneurium. The axis is stained but the myelin sheath is unstained thus, shows a clear area surrounding the axis.

5) Nerve bundle-spermatic cord

Examine the unstained nerve bundle running along with the arteriole and venule.

Practical 07: Bone and cartilage

Introduction

Bones are made up of specialized hard form of connective tissue consist of cells (osteoblasts, osteocytes and osteoclasts), collagen fibers and acellular calcified matrix (**osteoid**). **Osteoblasts** are bone forming cells which synthesize collagen and proteoglycan. The process of bone formation by osteoblast is called as **ossification.** Whereas, **osteocytes** are relatively inactive osteoblast cells which are found inside the **lacunae** (White colour fluid filled space) through which nutrients and gases diffuse in to the cells. O**steoclasts** also called as bone destroying phagocytic cells which breaks down bone matrix for bone remodeling and to release of calcium. The deposition of inorganic calcium phosphate as hydroxyapatite crystals within the matrix is a distinguishing characteristic of bones. The human skeleton made up of 206 bones provides the rigid protective supporting framework for the human body.

Macroscopically, there are two types of mature bones called as **spongy** (cancellous/trabecular) **bones** and **compact bones (lamellar)**. Spongy bones consist of small spaces filled with red marrow and forms the extremities (**epiphysis)** of long bones whereas, compact bones are relatively harder and consist of less spaces forms the long shaft (**diaphysis**) of the long bones. In addition, from outside to inside there are several layers ling one after the other in long bones. The outermost fibrous connective tissue covering layer of the diaphysis is **periosteum** beneath which compact bones are ling. Spongy bone lies beneath the compact bones and innermost yellow bone marrow cavity is lined by the delicate layer called **endosteum.**

Bones also exist as immature **woven bones** form with randomly arranged collagen fibers in prenatal life or in the repair of bone fractures. In this type of bone the matrix immediately surrounding the osteoblast is called as **osteoid** and is not mineralized. Whereas, in mature compact bones collagen arranged in regular parallel bands to form **Haversian system** (**osteons**). Because of its calcified matrix there are two methods of preparing histological sections of bone tissue. Bones may decalcify with acid solutions before embedding and sectioning but the detail of matrix is not clear with this method. Therefore ground sections are used to study the lamellar and canalicular pattern of calcified matrix but lacunae may appear empty sometimes as cells are removed during preparation. It is necessary to grind dried bones to a thickness the permits the microscopic light to be transmitted.

Objectives

At the end of this practical student should be able to,

- describe the organization and components of the bone tissue.
- relate the structure of bones to its function.
- recognize three types of bone cells.
- recognize Haversian system in compact bone.

1) Ground bone - Transverse section-H & E

Identify the Haversian systems made up of concentric bony layers (**lamellae**) arranged around the central canal (**Haversian canal**) consists of blood vessels, lymphatic and nerves under low power objective. These neurovascular bundles are interconnecting with one another and with periosteum as well as endosteum through canals running perpendicular to the Haversian canals called **Volkmann's canals**. Focus the slide under 40 × to observe lacunae and canaliculi formed by osteocyte cell processors. Observe the colour card provided next to the microscope to study the Haversian system with inner and outer circumferential lamellae.

2) Decalcified bone - Colour card - H & E stain

Identify the outermost periosteum (P) and outer circumferential lamellae (OCL) beneath it. Note three Haversian systems (H) consisting blood vessels in central canal of each. Between adjacent systems, there are irregular interstitial lamellae (I) and osteocytes with dark nuclei indicated with (OC). Proteoglycan rich ground substances in outer circumferential lamellae demarcate the outer limits of Haversian systems with basophilic cement lines (CL).

3) Osteoblasts - Colour card - H & E

Note the active osteoblast (Ob) depositing new osteoid on bone surface. These cells are large, spindle or cuboidal shape cells with abundant basophilic cytoplasm due to its large quantity of RER and Golgi apparatus which indirectly sense the high rate of protein (type I collagen) and proteoglycan synthesis.

4) Osteoclasts- Colour card - H & E

These pictures show the large multinucleated osteoclasts (O/ Ocl) which are often lying in depressions reabsorbed from the bone surface call **Howship lacunae** (H)/ resorption bay. These cells are aid in bone remodeling in response to growth and calcium homeostasis by their response to parathyroid and calcitonin hormones. The surface of the active osteoclasts folded into irregular projections called as **ruffled border**.

5) Osteocytes – Electron micrograph

Note the osteocytes enclosed by empty lacunae and their long processors forming gap junctions between adjacent cell processors to form canaliculi through which they communicate with each other. In H & E sections these cells appear flat and elongated and their functions are to maintain structural integrity of mineralized matrix and to mediate the release or deposition of calcium during calcium homeostasis.

Cartilage

Introduction

Similar to bones cartilage is a specialized semi rigid connective tissue made up of cells known as **chondroblasts** which produce the ground substance (mixture of proteoglycan and chondrotin sulphate) and mature **chondrocytes** lie in **lacunae** singly or in groups as well as the **matrix** rich in collagen or elastic fibres. Cartilage is avascular therefore mature cartilage has very limited capacity to repair and regenerate, but its matrix is permeable to nutrients and waste. Most mature cartilage has fibrous surrounding layer called as **perichondrium** made up of parallel collage fibres and few spindle shaped nuclei of inactive fibrocytes.

During fetal stage cartilage serve as a model for the development of cartilage/ endochondral bones. In adults cartilage act as a cushion in between articular surfaces of the joints and form ear pinna, nasal septa and supporting rings and plates of the trachea, bronchi and intervertebral disks. There are three types of cartilage depending on the type and amount of fibres in each.

- Hyaline cartilage
- Elastic cartilage
- Fibrocartilage

Objectives

At the end of this practical student should be able to,

- recognize the different types of cartilage.
- list the regions in the body where different types of cartilage are located.

6) Hyaline cartilage - Trachea - H & E

Hyaline cartilage is the most common type of cartilage. Do not move the slide. Change the magnification and study the appearance of the cartilage under the microscope. At high magnification locate the perichondrium surrounding the cartilage. This merges with the cartilage from one side and with connective tissue from the other side. Note chondroblast located adjacent to the perichondrium which are not yet embedded in the matrix. Observe the chondrocytes located inside the lacunae surrounded by matrix and arranged in two/three cell clusters. Note the cartilage matrix is relatively homogenous and basophilic. Other than in trachea hyaline cartilage also found in articular surfaces of movable joints, nasal septum, larynx, bronchi and in the sterna ends of the ribs and epiphysial plate of long bones. Go through the colour cards as well.

7) Elastic cartilage - Colour card

Elastic cartilage provides support with flexibility and found in external ear, external auditory canal, epiglottis and walls of Eustachian tube. The fresh elastic cartilage is yellowish in colour. Histological structure is more or less similar to hyaline cartilage except for the presence of numerous bundles of branching elastic fibres over collagen fibres in the matrix.

8) Fibrocartilage - Colour card - H & E

Fibrocartilage considered as a transitional type of tissue between hyaline cartilage and dense collagenous connective tissue. Note the dense collagenous connective tissue arranged in bundles. Small groups of lacunae containing cells are found in between the bundles of collagen. Found in regions where support and great tensile strength are desirable such as intervertebral discs and pubic symphysis. Refer on endochondral ossification, intra-membranous ossification and epiphyseal growth plate.

This finishes the practicals on four basic tissue types and rest of the practicals are designed to study their interactive functions in combinations as organs.

Practical 08: Skin

Introduction

The skin is the largest organ in the body both in weight and surface area. It is also known as **integument.** The skin is composed of an outer stratified squamous keratinized epithelial covering the **epidermis**, and the underlying connective tissue known as the **dermis**. The **hypodermis** or **subcutis** is made up of loose connective tissue and adipose tissue, located beneath the skin which anchors skin to the underlying structures. Additionally, there are specialized skin structures such as glands, nails and hair follicles which are mainly occupy the dermis and superficial subcutis.

The majority of the skin is thin skin which is mostly hairy and the thick skin is restricted to the ventral surface of the hands and feet. The skin provides protection against dehydration, abrasions, invading microorganisms (Physical barrier), and harmful effects of UV light. In addition, it acts as a sensory organ as it contains receptors for heat, cold, touch, pressure and pain (nociceptors). Other functions of the skin are synthesis of vitamin D, excretion of waste such as salt, ammonia and thermoregulation by sweating, piloerection/ pilorelaxation, skin vasodilatation/ vasoconstriction.

Objectives

At the end of this practical student should be able to,

- describe characteristic histological features of the different layers.
- relate the structure of skin with its function.

1) Epidermis - Transverse section - H & E

The epidermis is a continuously proliferating stratified squamous epithelium which has different layers with non-living surface layer of protein called as keratin. The basal layer is the **stratum basale,** the deepest layer composed of a single layer of cuboidal to low columnar cells in contact with basement membrane. Mitosis of these cells continuously replaces the cells loose at superficial layer. While differentiating these cells are moving bottom to superficial layers of the epidermis. Additionally, it contains the melanocytes (produce melanosomes consist of melanin pigment) and Merkel cells (tactile epithelial cells).

Focus the slide under low power objective to locate dermal-epidermis junction and focus under high power objective. Find the margin between light staining dermis and darkly stained epidermis to locate stratum basale. Screen this layer to find cells with mitotic figures. Note the presence of melanocytes with brown colour cytoplasm in this slide.

The basal cells are mature into form **stratum spinosum** which is several cells layers in thickness and forms the majority of the epidermis. These cells are attached to each other with intercellular bridges (desmosomes) giving spiny appearance to the cells. Note the polyhedral cells with centrally located round nuclei and prominent nucleoli in stratum spinosum. **Stratum granulosum** is consist of three to five layers of flatten cells. These cells are easily recognizable due to numerous dense basophilic keratohyalin granules in its cytoplasm. Locate dark staining stratum granulosum on top of the spinosum. Then observe the outermost **stratum corneum** consist of flattened anucleated keratin filled keratinocytes. The **stratum lucidum** found only in thick skin which is an intermediate stage where nuclei and cytoplasmic organelles are replaced with keratin protein. How many of these layers can be recognize in your class slide?

2) Langerhans cells - Colour card - H & E stain

Intra-epidermal antigen presenting cells. Present in all the layers of epidermis particularly around small blood vessels. Consist of clear cytoplasm, irregularly lobulated nuclei and cytoplasmic processors (CP).

3) Melanocytes - Colour card - H & E stain

These cells produce the melanin pigment responsible for skin and hair colour. The pigment presents in various forms from yellowish brown to black and has protective function against UV light. Present as separate individual cells in the basal layer of the epidermis.

4) Dermis- H & E

The dermis has a **papillary layer** and a **reticular layer.** The papillary layer includes the ridges and papillae that protrude into the epidermis: It is a loose connective tissue layer. The reticular layer is thicker and is made up of dense irregular connective tissue. This connects the dermis with the hypodermis. Try to identify these two layers in your class slide.

5) Hypodermis - Colour card - H & E

The hypodermis contains larger vessels which supply and drain the dermal vasculature. The loose connective tissue contains collagen and elastic fibres. The hypodermis also contains adipose tissue. Identify the type of adipose tissue.

6) Hair - H & E

In the skin, hairs are located in hair follicles. The hair follicles are well demonstrated in this slide in longitudinal section. These are located in the dermis and may extend into the hypodermis as well. The part of the hair protruding out of the follicle is the **shaft** and the part of the hair within the follicle is known as the **root**. The root has a hollow knob, the bulb to which a dermal papilla is attached. Identify these structures. The follicle has four major components- the **medulla**, **internal root sheath, external root sheath** and the **dermal papilla**. Which of these structures can you recognize? Medulla consists of loosely arranged keratinized cuboidal cells and it is surrounded by single layer of cells in cortex. Note the presence of sebaceous and sweat glands in close association with hair follicles.

Notice the presence of band of muscle in contact with hair follicles. This is the **arrector pili** muscle which aid in piloerection and pilorelaxation during thermoregulation. Is it a smooth muscle or a skeletal muscle?

7) Mammary gland- Colour card – H & E

Mammary gland is a compound tubuloalveolar gland consists of a capsule, interstitial connective tissue, secretory epithelium and a system of excretory ducts. Secretory units are grouped together to form lobules. The structure of the mammary gland shows much variation according to physiological status. Mammary activity commences in late pregnancy and continues throughout lactation. Resting mammary glands are seen in non-pregnant and non-lactating women.

- **Mammary gland (Active) -** Colour card – H & E

The parenchyma consists of alveoli and secretory tubules. Note the presence of eosinophilic secretory material in the lumen and vacuoles in some alveoli which represent the extracted lipids.

Note the epithelium of the alveoli, secretory tubules and ducts. Identify the myoepithelial cells associated with the alveoli and secretory tubules. Note the interlobular ducts lined by an epithelium with 2 cell layers.

- **Mammary gland (Inactive) -** Colour card – H & E

Notice the presence of much connective tissue and little parenchyma. Most of the epithelial tissue seen belongs to the duct system. The lining of the ducts changes from a simple cuboidal to a two layered epithelium. Alveoli are rudimentary. The connective tissue stroma is abundant and contains adipose tissue.

Practical 09: Histology of Lymphoid Tissue

Introduction

Lymphoid tissue is a specialized connective tissue consisting of a framework of reticular tissue with reticular fibers, reticular cells and free cells which are mainly lymphocytes. Lymphoid tissue arranged into one of the followings,

- **Diffuse lymphatic tissue**
  - *Lymphocytes are scattered diffusely in loose CT*
  - *No capsule present*
  - *Found in almost all the organs*
- **Lymphatic nodules**
  - *Spherical masses*
  - *No capsule present*
  - *Found singly or in clusters*
- **Lymphatic organs**
  - *Capsule present*
  - *Lymph nodes, spleen, thymus*

Objectives

At the end of this practical student should be able to,

- recognize lymphoid tissue in any body organ.
- distinguish between diffuse and nodular lymphoid tissue.
- recognize characteristic histological features of lymphoid organs under the microscope.

1) Thymus -H & E

Note the presence of outer capsule and septa. Screen the whole slide under low power objective. This section consists of several lobules and note the **cortex** and **medulla** in each lobule. Outer cortex is more basophilic whereas inner medulla comparatively eosinophilic and pale staining. Cortex is consists of closely packed immature/maturing lymphocytes and pale staining macrophages. These cells are also called as **thymocytes**. A few reticular cells are also present. Maturing thymocytes are migrating towards the medulla and loosely packed. The characteristic histological feature in the thymic medulla is the presence of **Hassall's**

corpuscles. These are acidophilic spherical structures composed of concentrically arranged keratinized epithelial cells. The center of the corpuscle is often degenerating. During involution, the demarcation between the cortex and medulla is lost and the thymus is infiltrated with adipose tissue.

2) Lymph node- H & E

Screen the whole slide under low power objective. Note the thick connective tissue **capsule** covering the lymph node and the **trabeculae**. Identify the outer **cortex** and inner **medulla** under low power. Note the presence of secondary lymphatic nodules with pale staining germinal centers in the cortex. Focus the secondary nodule under high power to observe lymphocytes with smaller and deeply stained nuclei at the periphery and pale staining bigger lymphoblasts in the germinal centers.

In the medulla, the dense lymphoid tissue does not arrange into nodules. Medulla is composed of extension of cortical masses called as **medullary cords**. The spaces in-between cords act as interconnected lymphatic channels called as **medullary sinuses**. Additionally, there are **subcapsular sinuses** just beneath the capsule.

3) Spleen-H & E

Examine the slide under low power objective and note the outermost thick **capsule** and **trabeculae**. The splenic parenchyma consists of **red pulp** and **white pulp**. The red pulp made up of venous sinuses filled with blood and white pulp is made up of lymphatic nodules.

Q1) are there germinal centers in these nodules?

Q2) how do you distinguish a section of a spleen from a section of a lymph node?

4) Palatine tonsils-H & E

The palatine tonsils consist of an aggregate of lymph nodules and diffuse lymphoid tissue. It is separated by underlying muscle by a dense collagenous **hemicapsule**. The luminal surface is covered by stratified squamous epithelium. This epithelium deeply invaginates into the tonsil in certain places, forming blind ended **tonsillar crypts.** Lymphoid nodules contain germinal centers.

Practical 10: Circulatory system

Introduction

The circulatory system, also known as the cardiovascular system consists of heart and blood vessels. The vessels that carry blood from the heart to the tissues are the arteries. In the tissues, they terminate in the microvasculature consisting of arterioles, capillaries and venules. Blood is returned to the heart from here by veins.

The basic structure of a blood vessel wall consists of three layers. From inside to outside, these are tunica intima, tunica media and tunica adventitia. Depending on the type of blood vessel, the number of layers and the amount and nature of tissue in each layer varies.

Objectives

At the end of this practical student should be able to,

- identify the different types of vessels in the circulatory system.
- relate the structure of vessels to its function.

1) Aorta -H & E

The main vessel that conducts the blood away from the heart is the aorta. Examine the slide and identify the layers and the endothelium which is the innermost layer of the tunica intima. Note the **vasa vasorum** the small blood vessels in the wall of the aorta to supply nutrition to the tunica media and tunica externa.

2) Arteries and veins- H & E

Examine the artery and vein focused in two different microscopes. Distinguish artery from a vein. What criteria you would use for this purpose?

3) Arterioles-H & E

The smallest arterial vessels are known as arterioles. Examine the spleen slide to identify arterioles. They have prominent endothelia and one or two layers of smooth muscle in the tunica media.

4) Capillaries-H & E

These are smallest of the vessels and they have only the endothelium. These are the vessels that permit materials to pass across the wall. This is known as capillary permeability. Examine the electron micrographs. Identify the structural features associated with permeability.

Practical 11: Endocrine and Exocrine Glands

Introduction

Glands are specialized epithelia that are having secretory function. These glands are made up of invaginations of surface epithelia into the deep tissues. While the most glands are multicellular there are unicellular glands as well like goblet cells. There are two types:

- **Exocrine**: Connected to the epithelial surface by a duct system
- **Endocrine**: Loose the connection with epithelial surface and lacks duct system

Exocrine glands are classified according to the morphology of the gland under histological sections.

- **Duct system** (branched/unbranched)
- **Arrangement of secretory component** (coiled/branched)
- **Shape of secretory component** (tubular/acinar)

Acinar type exocrine glands further divide into three categories depending on their type of secretion.

- **Serous secretion** (Parotid gland)
- **Mucus secretion** (Sublingual salivary glands)
- **Mixed both serous and mucus** (Submandibular gland)

Endocrine glands are classified into three types of depending on their location and major function.

- **Major endocrine organs** (e.g. thyroid, adrenal)

- **Endocrine components within other solid organs** (e.g. pancreas, ovary, testis and kidney)

- **Diffuse endocrine system (**e.g. GI and respiratory tracts)

Endocrine glands are composed of islands of secretory epithelial, supporting tissue and rich in blood capillaries. In addition, cells of the endocrine system have prominent nuclei and darkly stained cytoplasm due to abundant RER, mitochondria and Golgi bodies in cytoplasm.

Objectives

At the end of this practical student should be able to,

- identify different types of exocrine glands.
- list the location of different types of glands in the body.
- name the type of secretion and its function.

Examine the following slides. Draw and label the characteristic features of each gland.

1) Large intestine- H & E - 4× and 10×

Identify the simple tubular glands in transverse section of colon. Glands are straight and composed of mucous secretory goblet cells. These cells line the entire duct as well thus it is difficult to separate ducts from the secretory portion.

2) Stomach-pylorus- H & E - 4× and 10×

Observe the slide under low power and high power objective. Note the mucous secretory cells lining the glands and pale staining circular cross sections of the branching tubular secretory portion located underneath the darkly stained ducts opening into the surface. Have a rough idea on number of cross sections that can be seen under low power magnification.

3) Skin- H & E

There are three skin slides. One demonstrates the sweat gland and the other demonstrates the sebaceous gland focus under oil and the third slide is free to move and change the magnification.

- **Sweat glands**: H & E - 40 ×

Note the simple cuboidal epithelium in secretory portion and stratified cuboidal epithelium in non-secretory ducts. Therefore, cross sections of ducts are appearing more dark purple in colour compared to cross sections of secretory portion under low power.

- **Sebaceous gland-** H & E - 40×

Note the characteristic mesh/net like appearance in sebaceous gland. Cell cytoplasm is poorly stained with the centrally located dark nuclei. Each gland is consist of several secretory acini.

Q1) Name the secretory product and its function.

Q2) State the mode of secretion?

- **Skin slide**

Move the slide and change the magnification. Note the location of each gland and close association of sebaceous gland with the hair follicles. Recall your knowledge on connective tissue and epithelia.

4) Brunner's glands of the duodenum- H & E - 10× and 40 ×

Observe the light staining mucous secretory Brunner's glands in the duodenal submucosa. Ducts are opening into the villi surface but rarely seen. Note the number of cross sections under low power and compare it with the number of cross sections that can be seen in pylorus slide.

Q1) Why do compound glands have more cross sections compared to simple glands?

5) Pancreatic acini- H & E - 40×

Observe the lobulation under low power objective. Note pancreatic acini composed of cluster of secretory cells which are basally basophilic and apically eosinophilic/acidophilic. Observe the shape and the location of the nuclei. Identify the lining epithelium of the duct at the pointer.

Q1) What type of gland is this?

Q2) What is the reason for this characteristic staining feature?

6) Submandibular salivary gland- H & E-oil

Focus the slide under low power to observe the capsule around the gland and septa dividing the whole gland into lobules. At the center of the section you will be able to see bigger ducts. Then focus the slide under 10× and observe the pale staining cross sections of mucous secretory acini and darkly stained cross sections of serous secretory acini/tubular secretory portion. Due to the presence of both acini and tubular secretory portions it is named as compound tubule-acini glands. Certain mucous secretory acini are lined with small portion of serous secretory part call serous demilunes (SD). Note the mucus acini with foamy cytoplasm and flattened basally located nuclei, serous acini with circular basally located nuclei, serous demilunes and lining epithelium of ducts in the focused slide.

Q1) What type of gland is this?

Q2) State the reason for different staining properties of mucous and serous acini.

7) Parotid gland- H & E

Focus the slide under low power and screen the whole slide. Note darkly stained serous acini separated by thick septa and ducts going through the septa. Identify the lining epithelium of the ducts. Focus the slide under high power to observe smaller ducts scattered in-between acini and adipocytes filling the spaces between acini.

8) Sublingual gland- H & E

You will see pale pink staining and slightly dark staining two areas in the gland under low power. That has happened due to the staining error. Focus comparatively pale pink staining area under high power to observe poorly stained foamy cytoplasm in serous acini.

9) Hypophysis- H & E

Observe the slide under low power and identify the light staining posterior pituitary, comparatively dark staining anterior pituitary and pars intermedia in between. Pars intermedia is dark purple stained and consist of colloid filled vesicles. Then focus anterior pituitary under ×40 and recognize chromophils with darkly stained cytoplasm and chromophobes with poorly stained cytoplasm. Observe the fibrous nature due to the presence of axons of neurosecretory neurons and dispersed nuclei of supporting pituicytes. **Herring bodies** are not clear in this class slide.

Q1) What is the hormone secreted from pars intermedia?

Q2) Why AP more darkly stained compared to PP?

10) Thyroid gland- H & E

Focus the slide under low power and observe the follicles congaing eosinophilic, homogenous colloid at the center. Note the connective tissue septa dividing the whole gland into lobes and lobules. Focus the slide under oil immersion to see pale staining parafollicular cells lay in between follicles singly or as clusters.

Q1) Mention three hormones secreted by the thyroid gland.

Q2) State their functions.

11) Parathyroid gland- H & E

Observe the slide under low power to see its close association with the thyroid gland and the thin capsule. Glandular cells arrange into clusters and stain with basophilic dye. Focus the slide under oil immersion to see small principal/chief cells with round central nuclei and pale or clear cytoplasm. There is another type of cell which are comparatively bigger and having copious eosinophilic cytoplasm. These cells are called as oxyphil cells.

Q1) Which of these two cell type secretes the PTH?

12) Adrenal gland (Rat)- H & E

Identify the cortex and medulla under low power objective. Study the following structures in the medulla.

- Prominent vein located in the centre of the medulla
- Clusters of polyhedral cells with granular basophilic cytoplasm
- Numerous capillaries in the stroma

13) Adrenal gland (43-2)- H & E - oil

Observe following characteristic features in the different layers of the cortex under oil immersion. There are no clear demarcations between different layers.

- **Zona glomerulosa**
  Lying beneath the **thick capsule**
  Secretory cells arranged in rounded clusters
- **Zona fasciculata**
  Parallel cords of secretory cells with pale cytoplasm. Broader layer at the middle.
- **Zona reticularis**
  Lies adjacent to the medulla
  Small closely packed cells arranged in irregular cords

Q1) Mention hormones secreted by each cortical cell layer.

14) Endocrine pancreas- H & E - 40×

Observe the Islets of Langerhans focused under low power and note the small pale staining glandular cells in cluster with prominent dark purple nuclei at the center. Then focus the area

under high power and observe beta cells with pale staining cytoplasm and alpha cells with darkly stained cytoplasm.

15) Pineal gland- H & E - oil

Observe the **Pinealocytes** arranged into clusters with large round nuclei, prominent nucleoli and granular cytoplasm and small darkly stained nuclei of **Neuroglial cells** dispersed between pinealocytes. Note basophilic extracellular **pineal sand** at the pointer.

Practical 12: Respiratory system

Introduction

The function of the respiratory system is to provide oxygen to the tissues and eliminate carbon dioxide from the tissues. There are two functional zones, named as conducting zone which removes the dust particles, warm and moisturize the inspiratory air while respiratory zone which contribute for the actual gas exchange.

- **Conducting zone**: nasal cavity, nasopharynx, larynx, trachea, bronchi, bronchioles
- **Respiratory zone**: Respiratory bronchiole, alveolar ducts and alveoli

Bronchi, bronchioles, alveolar ducts and alveoli located within the lungs. Blood air barrier of the lung is made up of endothelial cells of capillary, fused basement membrane and simple squamous epithelial cells of the alveoli is acting as a barrier to pathogens and permits effective gas exchange at the lungs.

Objectives

At the end of this practical student should be able to,

- identify histological features of trachea (Epithelium, cartilage, trachealis muscle and glands).
- differentiate trachea, bronchi, bronchioles, respiratory bronchioles using histological features.
- list the functional adaptations of different regions of the respiratory tract.
- identify peumocyte I, pneumocyte II, dust cells and blood air barrier in lung histological sections.

Examine the following slides. Draw and label the characteristic features of each under low power and high power objective.

1) **Larynx-** H & E - 4× 10× and 40×

Move the slide and change the magnification. Identify hyaline cartilage and muscles in close association. Cartilage has damaged during preparation thus there is a white colour space at the top. Note the detached mucosa and submucosa at the center. Focus this area under high power objective and observe the lining respiratory epithelium and seromucous glands in submucosa.

2) **Trachea(rat)-** H & E-×4 ×10 and ×40

Screen the whole slide under low power objective first. Observe the muscle bundle outside the trachea. Identify the muscle type. Note the C shaped cartilage and trachealis muscle connecting free ends of the cartilage under low power. Respiratory epithelium is detached from the rest of the underlying layers in certain areas. Cartilage also not continuous C shaped. Then observe the cartilage, trachealis muscle and respiratory epithelium under high power objective. Note the thick band of fibroelastic tissue in lamina propria. Submucosal glands are not clear in this section.

3) **Trachea (Japan slide)** H & E - 4× 10× and 40×

Observe the slide under low magnification. Whole tracheal section cannot be seen at once even under low power objective because the section is bigger. Note the wide cartilage layer. Spaces in the cartilage are due to the preparation errors. Then move the slide along the cartilage until you see the blunt end of cartilage. Detect the next blunt end as well. Focus this area under high power and observe the layer of smooth muscle directly underneath the respiratory epithelium (RE). This is trachealis muscle comparatively longer than in previous section. Move the slide between two cartilage ends to see the ends of trachealis muscle.

Then move the slide and focus area away from the trachealis muscle and look for the mixed seromucous glands in submucosa. Note dark purple staining pseudostratified ciliated columnar epithelium lining the lumen.

Q1) What is the function of C shaped cartilage and trachealis muscle?

4) **Lung (Japan slide)-** H & E - 4× 10× and 40×

First focus the slide under ×4 and screen the whole slide from one end to another. Note scalloped horizontal edges of the section and irregular cut edges on top and bottom. Focus the scalloped edge under high power and observe mesothelial cells of the visceral pleura. (simple squamous cells with elongated nucleus)

Focus the slide under ×4 again and try to located following structures.

- Bigger elongated pulmonary vein
- Bronchus with irregular small cartilage plates, bigger lumen and lining RE
- Bronchial artery closely associated with the bronchus. Note thick tunica muscularis

- Two bronchioles in the section with their associated bronchiolar arteries: No cartilage plates at all. Note thick smooth muscle layer in the wall. Identify the lining epithelium.
- Observe the area just below the bronchus to locate respiratory bronchiole, alveolar duct and alveoli. Note the smooth muscles in broken respiratory bronchial wall and the simple cuboidal lining epithelium under high power objective. Observe the continuation of respiratory bronchiole as alveolar duct and opening of many alveolar sacs into single alveolar duct.

Q2) List the characteristic histological feature that can be used to differentiate bronchi from bronchioles.

Focus the lung slide under oil immersion to observe alveoli lined with flattened simple squamous epi cells and rich network of capillaries.

Type of cell	Description
Type I pneumocyte	Fairly thin cells with elongated nucleus covering more than 90% of alveolar surface. Frequently seen in histological sections. Form Blood Air barrier.
Type II pneumocyte	Fairly bigger circular cells with circular nucleus. Bulging into the alveolar lumen. Secrete surfactant which reduces the alveolar surface tension and prevents alveolar collapse during expiration.
Macrophage/dust cells	Locate inside the alveolar lumen as well as within the septum. Fairly bigger cells with foamy pale staining cytoplasm. Dust particles can be seen within certain dust cells. Clean the alveoli from invading microorganisms

Q3) What is the blood air barrier?

Q4) What are the structures that contribute to the formation of above barrier in lung?

Practical 13: Gastrointestinal system

Introduction

The function of the gastrointestinal system is to break down food for absorption into the body. The GI system is essentially a muscular tube lined by a mucous membrane that exhibits regional variations, reflecting the changing functions of the system from mouth to anus. The mucous membrane is protective, secretory, absorptive or a combination of these in different parts of the tract. The muscle gives strength to the wall of the tract as well as moving the food along. GI tract continue with the external environment therefore it is one of the potential portal of entry for pathogenic organisms. Thus the system incorporates a number of defense mechanisms which include prominent aggregations of lymphoid tissue, known as the gut-associated lymphoid system (GALT)

- **The alimentary canal**: consist of mouth, pharynx, esophagus, stomach, small intestine, and large intestine.
- **Accessory digestive organs:** salivary glands, liver, pancreas and gallbladder

Basic Histological Layers of the GI tract includes

- **Mucosa**
  Epithelium
  Lamina Propria
  Muscularis Mucosa
- **Submucosa**
  Loose CT, blood vessels, submucosal glands and submucosal plexus
- **Muscularis propria/externa**
  Inner circular, outer longitudinal and myenteric plexus
- **Serosa**

Objectives

At the end of this practical student should be able to,

- identify four basic layers of the GI tract in different regions.
- recognize different regions of GI system along with their functional adaptations.
- list the functions of different regions of the GI system .

Examine the following slides. Draw and label the characteristic histological features of each section.

1) Tongue slide 1- H & E - 4 ×

The tongue is a muscular mass, covered with a mucous membrane. Examine the slide under low power objective and note the foliate papillae with thin keratin layer on the tongue surface. Move the slide and observe the thick muscle mass underneath. Identify the muscle type. Observe the lower most covering layer of the tongue. Identify the epithelium precisely?

2) Tongue slide 2- H & E - 4×

Screen the whole section under low power objective. Note the conical filiform papillae on the tongue surface. These provide friction and roughness to the tongue. Note the thick keratin layer over the papillae. Move the slide while observing the papillae and note single circumvallate papillae surrounded by deep cleft at the back of the tongue. Taste buds are not clear on papillae in both sections. Examine and see whether glands can be detected in between muscle fibers.

3) Oesophagus (Japan slide) H & E - 4× 10× and 40×

Oesophagus is a strong muscular tube in which mucosa is folded into the lumen during relaxed stage. This section contain only a small portion of large esophageal cross section thus it appears to be longitudinal section. Note the stratified squamous non keratinized lining epithelium on the top, lymphoid aggregation in lamina propria and thick muscularsis mucosa. Screen the whole slide under low power objective to detect mucous glands, nerve bundles and blood capillaries in submucosa. Examine inner circular and outer longitudinal muscle layer below submucosa. Identify the muscle type.

4) Oesophagus class slide- H & E - 40×

This section also resembles the previous section. Note four basic layers in this section and prominent submucosal glands and their ducts in submucosa. Focus the pale staining thin layer of myenteric plexus in between inner circular and outer longitudinal muscle layers.

Q2) How does the muscularis externa change from top to bottom with reference to the muscle type?

5) Stomach (pyloro-duodenal junction)- H & E - oil

Screen the slide from one end to the other under low power objective. Start from your left side and note the section of duodenum with small villi, thin muscularis mucosa layer and prominent mucus secretory **Bruner's glands** in submucosa. Thickness of the muscularis externa is low. Inner circular layer has been cut transversely while smooth muscles in outer longitudinal layer have been cut longitudinally. Next examine the projecting muscular mass present as a continuation of the duodenum. This is pyloric sphincter located between duodenum and the stomach. Note the thick smooth muscle mass underneath the mucosa. Examine the area after the sphincter and note thick mucosa filled with gastric glands. Note prominent muscularis mucosa and identify the different types of cells in gastric glands. The surface epithelial cells are columnar and mucus secretory cells therefore show foamy cytoplasm. The luminal surface of these cells has a short brush border. Mucous neck cells have the similar appearance. Identify the dark purple staining **chief cells** with granular cytoplasm and basally located circular nuclei at the base of the gland. There are protein secretory cells. **Parietal/oxyntic cells** are scattered but more numerous in the neck region. These cells are recognized by their larger size, eosinophilic cytoplasm and centrally located circular nuclei. Muscularis externa in the pyloric region is comparatively thicker than that of the duodenum.

Q1) What type of glands is there?

6) Duodenum- H & E

Screen the slide under low power. Observe short villi in the mucosa lined with simple columnar epithelium. See whether goblet cells are present in the epithelium/ not. Examine the Bruner's gland in submucosa. In certain places glands are extending into the lamina propria as well. Note poorly stained foamy appearance and basally located flattened nuclei of the glands. Also note **Crypts of Lieberkuhn**, short intestinal glands located in between villi. Focus the Crypts of Lieberkuhn under high power objective to detect **Paneth cells** with intensely eosinophilic apical cytoplasmic granules. These cells contain antimicrobial peptides and protective enzymes such as lysozyme and aid in innate immune response. Compare the thickness of the wall when moving towards the large intestine.

7) Jejunum- H & E

Under low-power view of jejunum shows numerous circular folds with many shorter villi and crypts in cross sections, giving them a circular appearance. Observe the absence of Bruner's glands in submucosa. Under high-power objective note the simple columnar lining epithelium with occasional goblet cells and prominent microvilli (brush border). Note again the thin muscularis externa of the wall.

8) Ileum- H & E

Examine the slide under low power objective and observe tall circular folds with numerous villi. Note very thin muscularis mucosa and loose CT in the submucosa. Focus the villi under high power objective to observe highly abundant goblet cells in between simple columnar epithelial cells and the brush border. Note thick inner circular and outer longitudinal smooth muscle layers.

9) Colon- H & E

The LI consist of caecum, colon and the rectum. These three regions are difficult to distinguish histologically from one another. But it differs from SI in many ways.

- Absence of circular folds. Instead there are longitudinal folds
- Absence of villi and microvilli
- Presence of two types of cells
  Absorptive cells: surface columnar
  Mucus-secreting goblet cells: numerous, line simple tubular glands

Note thin mucosa and muscularis mucosa as well as thick muscularis externa arranged into inner circular and outer longitudinal.

Q1) What is the functional significance of this increased number of goblet cells?
Q2) What is the special stain that can be used to stain goblet cells? Mention the colour.

10) Appendix- H & E - 10×

Small, blind-ended, tubular sac extending from the caecum just distal to the ileocaecal junction. Move the slide and examine the whole cross section. General structure similar to LI. Characteristic feature is the presence of masses of lymphoid tissue in M & SM

11) Liver (three slides)- H & E

Examine all the slides. Note hepatic lobules and central vein under low power. Observe the cords of polyhedral hepatocytes with centrally located circular nuclei, prominent nucleoli and eosinophilc cytoplasm with basophilic granules under high power objective. Also note pale staining hepatic sinusoids in between cords. Identify the endothelial cells lining the sinusoids and the central vein. In third slide observe the portal triad consist of hepatic artery, portal vein and bile duct. Note simple cuboidal epithelium of the bile duct, large irregular shape of the vein with thin wall and comparatively small circular artery with thick muscular wall.

Q1) In to which major vein does the central vein drain?

Q2) What is the direction of blood flow in the sinusoids?

Q3) Name the cell type in liver that belongs to mononuclear phagocyte system.

12) Pancreas- H & E

Move the slide and observe the lobules, interlobular dusts, intralobular ducts, pancreatic acini and Islets of Langerhans. Try to locate centroacinal cells located at the center of the acini. These cells frequently contain one or more pale colour nuclei. Which region of the acini cells are basophilic and which regions are acidophilic?

13) Gallbladder- H & E

Muscular sac lined by a simple columnar epithelium. Mucosa branch and folds in non-distended state. Muscularis externa arranged in to different directions. Note outer thick collagenous adventitia.

Practical 14: Female Reproductive System

Introduction

The organs of the female reproductive system include ovary, oviduct, uterus, vagina, vulva and the mammary glands.

Objectives

At the end of this practical student should be able to,

- recognize different follicular stages in the ovary.
- identify the epithelia and their surface specializations in different locations of the tract.
- list the functions of different parts of the reproductive system.
- distinguish proliferative and secretory stages of the endometrium and list histological features.
- identify histological sections of active and inactive mammary glands.

1) Ovary (Japan slide)- H & E

This section is having many different follicular stages scattered throughout the slide. Therefore, screen the whole section under low power objective and identify the cortex and presence of blood vessels on right side corner (A). Move the slide from there while observing the edge of the ovarian cortex. Focus the cortical region under high power objective to observe thick fibrous capsule (**Tunica albuginea**) and outermost tall **mesothelial cell** layer.

Observe the location (B) under the power × 10 and × 40 to observe cluster of **primodial follicles** contain primary oocyte surrounded by single layer of flattened **granulosa cells (GC)**. There are few **primary follicles** as well. In these oocyte is surrounded by single layer of cuboidal cells. Identify the nucleus and nucleolus as well as cytoplasm of the oocyte. Certain follicles do not contain oocyte and certain oocytes do not contain nuclei at the middle. This is because the cutting section has not gone through the oocyte/nuclei. Examine the adjacent location (C) to examine advance stage of primary follicle. Note several layers of cuboidal GC and **zona pellucida (ZP)** a thick band at the periphery of the oocyte. Theca folliculi layer also can be seen outside the GC.

Then examine the area (D) to locate two **secondary follicles** nearby. Each contains oocyte surrounded by ZP and several fluid filled spaces in between GC layers. At this stage GC

appear circular with centrally located circular nucleus and clear nucleolus. Observe the Theca interna layer immediately outside the GC layer. It consists of dark purple staining roughly circular cells. Theca externa is the outer most layer consists of light staining elongated fibrous tissue and it blends with the surrounding CT stroma of the ovary.

Next focus the area (E) to examine two **Graafian follicle**s with single large fluid filled space and eccentric oocyte covered with cumulus oophorus and corona radiata cells. Note thin band of GC at the periphery. Focus under high power objective to study theca interna and externa. Move the slide over the section to detect white colour shrunken masses which are corpus albicans and try to locate degenerating follicles at different stages.

2) Ovary class slide- H & E

Examine the slide provided to you on the table with your naked eye. Same slide has been focused. Then screen the whole section under low power objective. Note the medulla consist of blood vessels and three big follicles with antrum in which oocyte cannot be seen. Examine the bigger **corpus luteum** with scallops' appearance due to the presence of fibrous septa. Focus the CL under high power objective to detect large polygonal shape Granulosa lutein cells with pale eosinophilic cytoplasm and round nuclei. Also observe the **atretic CL** in which there is lot of fibrous tissue, fibroblasts and fat infiltration can be seen.

3) Oviduct (Japan slide)- H & E

This cross section has been obtained from infundibulum region. Move the slide and examine the whole section under low power objective. Note highly folded mucosa and lots of blood vessels in one corner. Under the power × 10 to observe inner circular and outer longitudinal muscle layers. Outer longitudinal layer has highly invaded with blood vessels. Then focus the mucosa under ×40 to observe the epithelium. Note simple columnar ciliated cells as well as "non ciliated" secretory cells. The ciliated cells are generally shorter than the secretory cells, thus the epithelial surface somewhat irregular in outline.

Q1) List three functions of the Fallopian tube.

4) Uterine Endometrium (Proliferative phase)- H & E

Do not move the slide. Change the magnification only. Identify dark purple staining endometrium separately from light staining myometrium under low power objective. Note the low thickness of the endometrium and wider myometrium.

Focus the endometrium under ×10 to detect few cross sections of simple tubular endometrial glands. Secretory materials cannot be seen inside the lumen. Then observe the myometrium and note the smooth muscles arranged in different directions and presence of blood vessels in between.

Focus the epithelium under high power objective to observe ciliated simple columnar lining epithelium.

5) Uterine endometrium (secretory phase)- H & E

Move the slide and examine whole slide under low power objective. Note the thick endometrium on left hand corner of the section. Then thick pale staining myometrium beneath that and the outermost thin band of perimetrium and the serosa. Focus the endometrium under high power objective to observe numerous cross sections of coiled tubular glands filled with secretory material and the simple columnar lining epithelium of the glands. Compare the appearance and number of endometrial glands as well as thickness of the endometrium with previous slide.

Q2) What is secretes by endometrial glands and mention its function.

6) Uterus (Rat)- H & E

Screen the slide under low power. Observe the whole cross section of the uretus. Examine the endometrium. What is the phase of endometrium? Justify your answer. Then observe the inner circular and outer longitudinal smooth muscle arrangement. Generally it is irregularly arranged. Try to focus perimetrium and observe its components.

7) Cervix - H & E

Observe the slide provided to you with your naked eye. This is a part of external cervical orifice which protrudes into the vagina. Examine the slide under low power objective and observe the slightly folded mucosa on the top. Focus this area under high power objective to examine simple columnar epithelium on the surface. Note some ciliated and mucus secretory cells located in between. Then move the slide along the mucosa towards the right side. Examine the non-keratinized stratified squamous epithelium of the vagina.

8) Vaginal cuff- H & E

Move the slide and change the magnification. Note non-keratinized stratified squamous epithelium of the vagina and abundant elastic fibers in the lamina propria and submucosa.

Q1) List the functions of the vagina.

Practical 15: Male Reproductive System

Introduction

The organs of the male reproductive system include testes, epididymis, vas deferens, accessory sex glands and penis.

Objectives

At the end of this practical student should be able to,

- recognize certain cell stages of spermatogenesis in seminiferous tubules, Leydig cells, rete testes and efferent ductules.
- distinguish different histological features of head, body, tail regions of the epididymis.
- identify the different structures of the spermatic cord.
- differentiate different accessory sex organs histologically.
- discriminate corpus spongiosum, corpus cavernosum and urethra in a cross section of a penis.

1) Testes -H & E

There are three testes slides focus under three microscopes. Observe the provided slide beside the first microscope with your naked eye. There are two sections in the same slide. Then focus one section under low power objective and screen the whole section.

Note the thick testicular capsule/**tunica albuginea** made up of dense connective tissue on either sides of the section, numerous cross sections of **seminiferous tubules** and **rete testis** towards the upper end of the section. Observe the simple cuboidal epithelium of rete testis under high power objective. Try to identify above mentioned structures, draw and label only low power view.

2) Testes (Japan slide) - seminiferous tubules - (oil)

Do not move the slide or change the magnification. Examine the Sertoli cell focused at the tip of the pointer and note roughly triangular nucleus and the nucleolus. Identify the spermatogonia adjacent to the Sertoli cell and primary spermatocytes located more towards the lumen. Note the characteristic granular nuclear material of the primary spermatocytes.

3) Testes- Leydig cells - H & E- 40×

Do not move the slide. Observe the pale foamy cytoplasm of Leydig cells present as a cluster within the interstitium in-between three seminiferous tubules. Note roughly circular nucleus of it. Examine the surrounding cross sections of seminiferous tubules in order identify circular densely stained spermatids and the elongated sperms close to the lumen.

4) Efferent ductules- H & E

Do not move the slide. Change the magnification and light accordingly. Change the fine focus if necessary. Note the "non ciliated" short secretory cells with foamy cytoplasm and ciliated columnar epithelial cells. Thus, the outline of the lumen is not smooth due to the variation in height of the epithelium. Focus the slide under ×10 to observe the presence of smooth muscle and supporting CT around each efferent ductile.

5) Epididymis- head (Japan slide-28)- H & E

The epididymis consists of a single highly coiled tubule. Do not move the slide and observe the head of the epididymis under low power objective. Note the lumen size, presence of few sperms inside the lumen, height of the epithelium. The epithelium of ductus epididymis is pseudostratified columnar and composed of two types of cells. Focus the slide under high power objective to examine principal cells characterized by long stereocilia and smooth muscles around each cross section.

Q1) Describe the characteristic histological features of the epididymis that facilitates its function.

6) Epididymis (Body and Tail)-donated by Faculty of Veterinary Medicine

Do not move the slide. Tip of the pointer placed on the margin between body and tail regions. First observe under low power objective and compare the difference in epithelial height, length of the stereocilia, lumen size and amount of sperms inside the lumen. Focus the slide under 10× and compare previously mentioned facts again.

7) Spermatic cord- H & E

Move the slide and examine whole slide under low power objective. There are two segments separated from each other. The left sided segment consist of vas deferens, nerve bundle and

few cross sections of veins. The next segment consists of testicular artery surrounded by many cross sections of veins/pampiniform plexus.

Focus the vas deference under high power objective. Characterized by four layers- mucosa, propria submucosa, muscularis propria and serosa. The mucosa is thrown into folds and the epithelium is pseudostratified. Examine the inner longitudinal, middle circular and outer longitudinal smooth muscle layers. All three muscle layers are clear only on one side of the vas deference.

Q2) What is the function of pampiniform plexus?

8) Prostate gland- H & E

Screen the slide under low power to examine both lobes. Note the presence of secretory alveoli (branched tubuloacinar glands), septa and thick fibrous capsule at the periphery. Under ×10 note the tall columnar secretory epithelial cells with prominent round basal nuclei and pale-staining cytoplasm. Scanty population of small, flat, basal cells at the base of the gland in contact with the basement membrane.

9) Seminal Vesicle - H & E

This is a compound tubular or tubuloalveolar gland. The epithelium is Pseudostratified columnar. Note the highly folded mucosa and honey comb appearance under low power objective. Wall consists of inner circular and outer longitudinal smooth muscle layers. Examine the secretory cells with lipid droplets in cytoplasm (foamy) under high power objective.

10) Penis- Special stain

This slide has three cross sections taken at different levels. The penis is consist of three cylindrical masses of erectile tissue, dorsally located paired corpora cavernosa and ventrally located single corpus spongiosum. The corpus spongiosum encloses the penile urethra. Locate the urethra in each cross section. Note the presence of cavernous spaces in erectile tissue. In one cross section surface of the penis is covered with stratified squamous keratinized epithelium. Examine the penile artery and veins in certain cross sections.

Practical 16: Urinary System

Introduction

The urinary system consists of paired kidneys, ureters, urinary bladder and the urethra. The major functions of kidneys are excretion of metabolic waste and foreign chemicals as well as hormonal metabolites, regulation of water and electrolyte balance, regulation of arterial pressure, acid-base balance, erythrocyte production and vitamin D3 production. Rest of the organs involve with storage and outflow of urine.

The structural and functional unit of the kidneys is **nephron** which consists of **renal corpuscle**, **proximal convoluted tubule (PCT)**, **Loop of Henle** and **distal convoluted tubule (DCT)**. The renal corpuscle, PCT and DCT are located in the cortex while loops of Henle and the collecting ducts lie in the medullary rays and extending into the renal medulla.

Objectives

At the end of this practical student should be able to,

- differentiate renal cortex from medulla.
- identify renal corpuscles and differentiate different regions of tubules in a histological section.
- relate the histological adaptations in each region to its function.
- identify juxtaglomerular apparatus and state its function.
- recognize transitional epithelium and state its function.

1) Kidney - H & E

Examine the whole section under low power objective. Note the outermost thin capsule covering the kidney. Differentiate the cortex with renal corpuscles from the medulla with longitudinal cut sections of tubules. Renal corpuscles are consisting dense round **glomeruli** surrounded by white colour spaces (**Bowman's spaces**). This capsular space is bounded by inner (visceral) and outer (parietal) layers of Bowman's capsule.

Q1) Identify the lining epithelium of the Bowman's capsule.

The afferent and efferent arterioles enter and leave the glomeruli at the **vascular pole** of the renal corpuscle. Examine the renal corpuscle to identify the vascular pole. At the opposite

pole which is the **urinary pole**, the Bowman's capsule continues into the proximal convoluted tubule. If you search the slide hard enough you may be able to see this. Note the presence of many PCT and few DCT cross sections. PCT are lined by Simple cuboidal epi with brush border (microvilli) which makes the apical surface irregular. Thus, the lumen appears small. Epithelial cell cytoplasm is darkly stained due to high mitochondria content. DCT cross sections are seen among PCT sections. These also lined by simple cuboidal epithelium without a brush border. Therefore, lumen is larger and clearly defined. Number nuclei per cross section of DCT are higher compared to PCT and the cytoplasm also comparatively pale pink in colour.

Q2) List the functional adaptations in PCT.

Focus the medullary rays to examine the collecting tubules/ducts lined by either simple cuboidal/ columnar epithelium. Then focus the medulla to identify thin limb of Loop of Henle lined with simple squamous epithelium and the thick limb lined by simple cuboidal epithelium. Note the cross sections of vasa recta closely associated with these cross sections. Differentiate those from thin limb by the presence of RBC inside the lumen.

2) Kidney slide (Juxtaglomerular apparatus)- H & E

Do not move the slide or change the magnification. Close to the vascular pole look for the **macula densa** cells of the DCT. These are densely packed specialized epithelial cells characterized by an accumulation of nuclei and comparatively darkly stained cytoplasm. The afferent arteriole close to this region has modified smooth muscles cells of tunica media called **juxtaglomerular cells.**

Q3) What is the function of macula densa and juxtaglomerular cells?

3) Ureter- colour card

The wall comprise of three basic layers. Inner mucosa is lined by transitional epithelium to facilitate stretching when it filled with urine. Mucosa also thrown up into folds during relaxed state. The transitional epithelium has four to five layers and is surrounded by broad collagenous lamina propria. The muscularis mucosa has inner longitudinal and outer circular layers. The outer adventitia contains adipose tissue and some blood vessels.

4) Urinary bladder-H & E

The bladder is lined by transitional epithelium which is about six to eight layers in thickness. Mucosa thrown up into folds in relaxed stage. It has well developed muscularis propria composed of three layers, inner longitudinal, middle circular and outer longitudinal. Three layers are collectively called as **detrusor muscle**. Layers are often difficult to distinguish. Outer Adventitial contains arteries, veins and lymphatics.


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