

PCB 4023 – Cell Biology

Lab 2: Introduction to Histology / Epithelium

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N.B. Since this document is in “pdf” format, the URLs (web addresses) cannot be linked. To use them, simply highlight and copy the address and paste it into the address box of your browser.

Preparation assignment (to be completed before lab (1/18/02):

- 1) Kerr (1999) Chapter 1. The Cell (pp. 1-24). Skim this chapter lightly.
- 2) Kerr (1999) Chapter 3. Epithelium (pp. 39-59). Read this chapter carefully, paying particular attention to the information that will help you identify the types of epithelium using light microscopy.

Web resources:

N.B. There are several histology courses/reviews/tutorials on the web, most of them originating from a medical school, and thus grossly biased towards human anatomy. Time has not allowed me to evaluate them all, but a good one has been prepared by Drs. George and Pawlina of the University of Florida College of Medicine. For each topic there are both review modules (two for epithelia) and an on-line quiz module which provides feed-back on incorrect answers. Another nice feature is a glossary (“unit terms”) at the end of each review module. Of course, these modules may contain information that is not covered in this course and you will not be held responsible for any information presented there. However, you may wish to consider using them as additional preparation and/or review. The URL is as follows:

<http://www.medinfo.ufl.edu/year1/histo/index.html>

Know of any other web sites pertaining to epithelium that you have found helpful or interesting? E-mail me the link at condon@fiu.edu. and let me know what and why you found it informative and/or interesting.

I. INTRODUCTION

The laboratory work in histology will consist of microscopic observation of slides containing sections or smears of cells, tissues, or organs. The laboratory directions include the number of the slides in your collection that you are to observe. The slides have cells and tissue types which correspond to many of the photographs in your texts. The text may help you locate and identify particular structures; it also describes and correlates the cells and tissues seen under the microscope.

The slides are all of human material unless stated otherwise; the stain is indicated in abbreviated form; further reference to the stain can be found in the section on Biological Stains and Techniques.

II. BIOLOGICAL STAINS AND TECHNIQUES

Acid dyes are those that interact with basic proteins, while basic dyes are those that interact with acid proteins or other acidic substances. Acid and basic dyes are usually “aniline” or coal-tar dyes. The staining with these dyes or stains depends on the nature of the proteins of cells and tissues.

A protein staining with an acid dye is considered to be acidophilic. On the basis of their staining affinities, tissues and tissue components are generally termed as acidophilic or basophilic. Red blood cells, eosinophilic leukocyte granules, parietal cell granules of the stomach are acidophilic; chromatin, Nissl bodies, granules of basophilic leukocytes, cartilage matrix, etc. are basophilic.

<u>Acid Dyes</u>	<u>Basic Dyes</u>
Eosin	Methylene Blue
Orange G	Thionin
Aniline Blue	Cresyl Violet
Acid fuchsin	Methyl green
Trypan blue	Basic fuchsin
Methyl blue	Toluidine blue
	Crystal violet
	Azure A and B

Common histological staining and their appearance:

The Bodian method uses silver and small amounts of gold and copper for impregnating nerve fibers which appear purple to black.

Carmine is a natural dye from cochineal products; it stains glycogen a bright red.

Cresyl violet (C.V.) is a basic dye which is used for staining nucleoproteins, Nissl substance, etc. It has metachromatic properties for mucin and mast cell granules. (See metachromasia).

The Feulgen technique is specific for the demonstration of DNA (deoxyribose nucleic acid). In this method mild hydrolysis with HCl forms aldehyde groups on the DNA sugar but not on the RNA (ribose nucleic acid) sugar. The aldehydes then react with reduced basic fuchsin (Schiff's reagent) to form a red-blue (magenta) color. This is the Feulgen nuclear reaction for DNA.

The Giemsa stain (mod. Giem.) is methylene azure and eosin used for differential staining of bone marrow cells. Results are similar to Wright's blood stain.

The Gomori (Gom) technique is a modified silver method for reticulum. There are also Gomori techniques for demonstration of alkaline and acid phosphatase enzyme activity in tissues.

Hematoxylin and Eosin (H. & E.) are general histological stains. Hematoxylin stains nuclei blue while eosin stains cytoplasm pink.

Iron Hematoxylin (FeH) is a natural dye with a mordant of iron. It stains chromosomes, mitochondria, and elastic fibers. The active component is hematein extracted from logwood bark; it is bound to tissue components by means of a mordant, usually a metallic ion, such as iron.

Metachromasia is the property exhibited by certain biological compounds to change the color of such dyes as toluidine blue and thionin. For example, mucoproteins and mucopolysaccharides found in cartilage matrix and mast cell granules will stain red or violet instead of blue with toluidine blue. This apparently is caused by polymerization of the dye molecule.

Mallory's Stain (Mal) is used primarily for connective tissues. It contains three dyes: aniline blue, orange G, and azocarmine (or acid fuchsin). Connective tissues, in general, stain blue; muscle stains red; epithelium is red due to red nuclei; red blood cells are orange-red.

Orange G (OG) is an acid dye staining cytoplasm.

Orcein (Or), a natural dye obtained -from lichens stains elastic fibers dark brown and other connective tissue pale brown.

Osmic Acid (Os) is osmium tetroxide and is useful for demonstration of certain lipids because unsaturated fatty acids reduce osmic acid to a black compound. It demonstrates myelin sheath, Golgi apparatus, etc.

Phosphotungstic acid hematoxylin (Pt. H) is used to demonstrate muscle striations, centrioles, etc.

Picro-fuchsin (P-f) is a counterstain used with van Gieson's hematoxylin stain.

Silver salts are used to impregnate certain structures such as Golgi apparatus, reticular fibers, neuroglia cells, neurofibrils, etc. There are numerous modifications of the silver method.

Sudan III, IV, and Sudan black are soluble only in fats and are used to stain fat droplets.

Toluidine blue is a metachromatic basic dye.

Unna-Pappenheim stain uses pyronin and methyl green and chromatin green. It stains nucleoli red

van Gieson's (v.G.) stain for connective tissues is picro-fuchsin and hematoxylin. It stains nuclei and epithelia brown, collagenous fibers bright red, and elastic fibers and muscle yellow.

Vital dyes are a class of pigmented non-toxic substances taken up by phagocytic cells of the body. Examples are trypan blue and Nile blue sulfate. Carbon particles are also used in phagocytosis demonstration. Supravital staining is done by adding certain dyes to the medium of cells already removed from the organism. Dyes include Janus Green, neutral red, methylene blue.

Weigert's (W) elastic stain is resorcin and fuchsin. It stains elastic fibers dark blue.

Weigert's myelin sheath stain (Weig) uses Fe Hematoxylin preceded by potassium bichromate. Myelin sheath is dark blue.

Weil's stain is a modified Weigert's stain, staining myelin sheath dark blue.

Wright's blood stain uses eosin and methylene blue to differentiate blood cell types. The following staining results, but variations from the ideal, will occur:

Erythrocytes	cytoplasm is orange to pink; nuclei (if present) deep blue
Monocytes	cytoplasm is gray-blue with light red granules; nuclei, lilac with reticular chromatin
Lymphocytes	cytoplasm is pale "robin's egg" blue; nuclei, purple blue
Polynuclear neutrophilic	cytoplasm has red lilac granules; nuclei, blue leukocytes
Eosinophilic leukocytes	cytoplasm is blue with red granules; nuclei, blue
Basophilic leukocytes	cytoplasm has dense purple granules; nuclei, purple

III. EPITHELIUM

Histology (Gr, web (tissue) + word) is literally the study of tissues but is more broadly defined (*sensu latu*) as the study of cells (cytology), tissues (histology; *sensu strictu*) and organs (organology) in relation to their function. Often the term microscopic anatomy or microanatomy is employed.

Tissues are collections of similar cells and the intercellular substances around them. There are four basic types of tissue and vast majority of cell types can be classified as one of these four.

- 1) epithelia - continuous layers of cells with little intercellular space which line surfaces and form glands
- 2) connective tissue - cells embedded in intercellular substances (extra-cellular matrix); the matrix is typically abundant the cells less so.
- 3) muscles - specialized contractile cells with electrically excitable membranes
- 4) nervous tissue - (1) cells specialized for conducting and transmitting electrochemically mediated information (i.e., neurons) and (2) the cells which support those engaging in this activity (e.g., neuroglia or glia)

[N.B. There are however, a few cell types which do not fit neatly into this classification. For example, ependymal cells are nervous tissue which form epithelial-like sheets with epithelial adhesions. Myoepithelial cells are epithelial cells containing contractile proteins.]

Today we will begin our study of histology by examining epithelia. An epithelium can be defined as a continuous layer of cells bound together by a basement membrane with little intercellular space and which lines surfaces and form glands. Developmentally, epithelia are derived from all three primary embryonic ("germ") layers:

- ectoderm > skin, cornea, lining of oral and nasal cavities, lining of anus and associated glands
- endoderm > epithelia of the digestive & respiratory tracts (including accessory organs: liver, lungs, pancreas) and associated glands
- mesoderm > epithelia of the urogenital system and associated glands **and**:
 - 1) mesothelia > lining of pericardial, pleural and peritoneal cavities
 - 2) endothelia > lining of blood vessels and lymphatic channels

Epithelia are broadly divisible into (A) coverings / linings and (B) glands. Covering and lining epithelia serve a variety of functions including:

- 1) forming selective barriers; e.g. skin, kidney, absorptive gut tract, lung alveoli
- 2) protection – to prevent dehydration and/or chemical and mechanical damage
- 3) sensory - the neuroepithelia (hair cells of hearing and balance, taste buds, olfactory epithelia)

Lining epithelia are classified on the following bases (see figure):

- 1) the number of cell layers between the basement membrane and free surface: simple (single) vs. stratified (2+) vs pseudostratified (see below)
- 2) the shape of the cells along the free surface: squamous, cuboidal, columnar

If the surface cells of an epithelium have apical specializations (e.g., microvilli, stereocilia, cilia, etc.), this characterization also is included in the classification, e.g., ciliated simple columnar.

Of course, many lining epithelia (e.g., the linings of the gut and respiratory tracts) have associated glands and thus also have a secretory function. In such epithelial, different epithelial cell types within the lining perform the different functions.

Glandular epithelia serve solely a secretory function and can be broadly divided into two major groups:

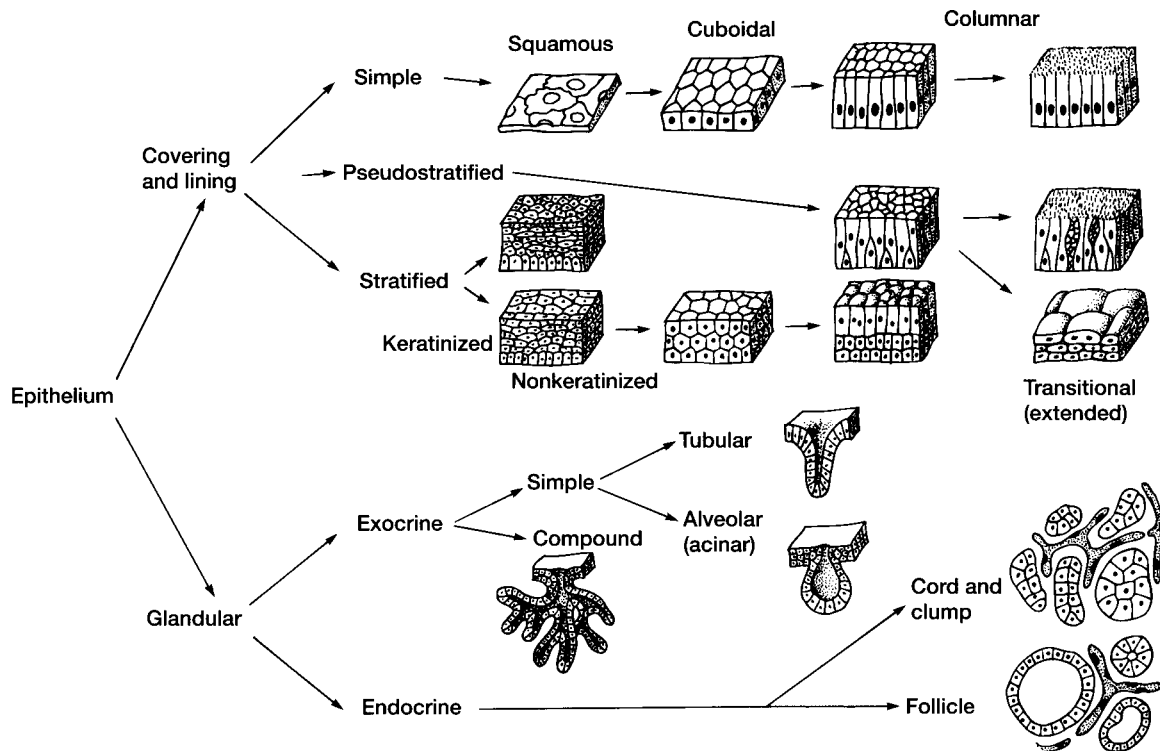
- 1) endocrine glands = ductless glands; in these glands the secretions are passed into the circulatory system.
- 2) exocrine glands = glands with ducts for secretion to a surface

Exocrine glands can be classified in a number of ways:

- 1) unicellular vs multicellular
- 2) duct morphology (multicellular only): simple (unbranched) vs compound (branching)
- 3) secretory cell arrangement: tubular, alveolar (acinar; flask-shaped) and tubuloalveolar;
- 4) secretions: mucous, serous (watery) and mixed (seromucous)
- 5) mechanism of release: merocrine (eccrine), apocrine, and holocrine

These classifications are described more fully in your text. Histologically, aside from the first classification (uni- vs. multicellular), it is difficult to determine duct morphology or secretory cell arrangement in a single section. Similarly, the nature of the secretions and mechanisms of release are not always apparent in histological section. Thus, the classification of the gland is usually supplied as part of its study.

figure: 3K5-18.tif



Lab Assignment: Epithelium

Work through the following sections using your atlas as a guide. Make sure to answer all questions marked by “?”. These will be evaluated when you turn in your handout next week. Items appearing in **bold** will form the basis for next week’s quiz.

To learn how to identify the structures, write down criteria which will assist you in your identification (e.g., simple squamous epithelium: single layer, flat cells with flattened nuclei). Your text is a good source for such material as well as your own observations. Some students find it helpful to make rough sketches of the structure to assist in their learning.

I. Simple Squamous

Simple squamous epithelium is very thin and flat. It forms the peritoneal lining (mesothelium) and the inner lining (luminal surface; endothelium) of all vascular elements (arterial, venous, capillary, lymphatic). Other examples of simple squamous epithelia include the alveoli of the lung and the Bowman’s capsule of the kidney’s nephron.

mesothelium (small intestine): HK 7-24
 endothelium (artery, vein and nerve): H 7040
 lung alveoli (lung): H 2460
 Bowman’s capsule (kidney): HA 1-3, H 3500

II. Simple Cuboidal

This epithelium lines small tubules and follicles and the cells are about equal in height and width with a central nucleus. It can be found most easily lining the collecting tubules of the kidney. Other examples include the lens capsule and some of the ducts in some exocrine glands (e.g., salivary glands).

renal tubules (kidney): HA 1-3, H 3500

III. Simple Columnar

This epithelium is found in organs adapted for absorption and secretion. The cells are taller than they are wide and the height varies a great deal. They are found throughout the lining of the gut (stomach, small intestine, colon). Note that in the duodenum the luminal surface shows columnar cells covering the villi (projections of the mucosa into the lumen). Many of them are striated on their free surface and scattered throughout-are goblet cells which are adapted for mucous secretion (see below). Other sites containing this epithelium include the uterine tubes and cavity and occasionally in the larger ducts of exocrine glands.

small intestine : HK 7-24, or 93W4526
 colon: 93W4542

IV. Pseudostratified Columnar

This epithelium contains columnar and basal cells and gives the appearance that it is layered. While all cells attach to the basement membrane, not all extend to the lumen. The pseudostratified columnar epithelia lining the upper respiratory tract (trachea and bronchi) are ciliated and contain scattered goblet cells (unicellular glands). Other tissues containing this epithelium include the male reproductive tract (epididymis, ductus deferens and portions of the urethra).

trachea: HJ 2-1
 epididymis (with testis): HM 1-2

V. Stratified Squamous

This is a thick epithelium found where protection is needed, such as body surface and body entrances. In the skin notice that the basal cells in the epithelium are rounded with a progressive flattening of the cells toward the free surface. When the surface is moist, as in the tongue, the free surface cells are not visibly cornified. Non-keratinized stratified squamous epithelia can also be found in the lining of the oral cavity (varies with species), esophagus, anal canal and vagina.

skin (keratinized): H 2081, or HI 1-3

tongue (non-keratinized): H2690

VI. Stratified Cuboidal and Columnar

These relatively rare types can be found lining large ducts (e.g., salivary, mammary), the pharynx, the palpebral conjunctiva, and the urinary passages. In the urethra the surface cells are tall and thus are stratified columnar. In salivary gland look for large, open ducts and notice that basal cells are low but the surface cells can be either cuboidal or columnar in shape.

urethra (prostate and urethra): 93W5472

sublingual gland: H2735; look for larger ducts

VII. Transitional

This unusual epithelium lines the urinary tract from the renal calyces through to a portion of the urethra, and the urinary bladder is perhaps the best example of this type. It is found in organs which undergo distention and therefore becomes thicker and thinner. Take note of the surface cells and compare them with the surface cells from stratified squamous as seen in the esophagus.

urinary tract (transitional epithelium): H 280

VIII. Epithelial Specializations

Goblet cells (gut and respiratory tract) are epithelial cells adapted for the secretion of mucous. The striated border of the duodenum are made up of many small finger-like projections called microvilli and these increase the surface area and are for absorption. Cilia (trachea) are protoplasmic processes which beat and move substances along their surfaces. The stereocilia seen in the epididymis are actually giant microvilli.

goblet cells: duodenum (HK 7-24 or 93W4526) , trachea (HJ 2-1)

microvilli - duodenum (HK 7-24 or 93W4526)

cilia - trachea (HJ 2-1)

stereocilia - epididymis (with testis; HM 1-2)

IX. Functions of Epithelial Cells

Epithelial cells are specialized for different functions. Protection results from layering or stratification. Pseudostratified ciliated cells function in transporting mucus and particulate matter along their surfaces. Epithelia which secrete, absorb, resorb and filter are single-layered, and their height correlates with their function.

X. Glands

As noted above, a glands classification (other than uni- vs. multi-cellular) is difficult to discern in a single section and thus is usually provided in the description. For this reason, we won't go crazy over glands but will look at a representative subset that can be observed in your text and slide box.

The simplest glands are the unicellular (i.e., glands consisting of one cell) and a good example is the goblet cell. Still simple, but multicellular, are the short tubular glands of the colon. An example of a compound acinar gland are the exocrine cells of the pancreas. Finally, an example of an endocrine cell will be examined (suprarenal).

unicellular goblet: small intestine (HK 7-24); colon (93W4542)

simple tubular: colon (93W4542)

compound acinar: pancreas (HK 9-2)

endocrine cells: suprarenal (HO 3-1 or H 3500)

XI. Questions

A list of questions and to be turned in Week 3 will be given in lab. Also a list of structures on which next week's quiz will be based will be provided.