Lab 14.

Microscopy I: Microscopy, Histology and Tissue Biology

I. Reading

Biology, 6th ed. by Campbell

Function correlates with structure in the tissues of animals p. 835
The organ systems of an animal are interdependent p. 839

II. Background: Philosophy, Cells, And The Microscope

During this period your goals are to:

- Observe histological sections with the compound microscope,
- Identify epithelial and connective tissue,
- Learn topology of representative epithelial organs.

The remarkable advances in molecular and cell biology are only the most recent and highly visible manifestations of a philosophy called “reductionism,” which guides much of modern inquiry in biology as well as the other sciences. “Reductionism” refers to the scientific explanation of a structure, process, or phenomenon in terms of structures or chemistry occurring at a lower level of organization. In this context, an “explanation” means a description of how something works. The smooth contraction of the heart, for example, is explained by the contractile properties of a special muscle cell and the biochemistry of muscle contraction. A molecular biologist would seek explanations for heart structure/function in terms of genes and gene regulation related to muscle structure and function. Reductionism is at the philosophical core of science and is, to many scientists, the soul of modern biology.

Why bring up a philosophical matter in an histology laboratory session? Because in the study of cells and tissues, both the origins of biological reductionism as well as its limits are unambiguous and dramatic. Rarely in this life do you get to meet a living philosophy face-to-face. Origins first. In 1665, Robert Hooke, after examining cork with a magnifying lens and noting its partitioned substructure, called the partitions “cells.” His contemporary, Antonie van Leeuwenhoek, also a pioneer in microscope design, discovered single-celled creatures in pond water. Hooke never realized the significance of his discovery, and it was not until 174 years later, in 1839, that the German biologists Matthias Schleiden and Theodor Schwann summarized their own findings and the microscopic studies of others by concluding that all living things consist of cells.

The discovery of a previously hidden level of living organisms led naturally to the reductionist concept of the cell as a unit of structure and function, a concept that to this day illuminates the search for new cell types. (Are new cell types really still being discovered? Try running a Medline search on “stem cells” and find out.) In
today’s laboratory session, you can, if you are in the right frame of mind, relive the experience of discovering cells and reflect on a new world.

Or maybe not. “Cells? Did that in high school.” Historical perspective is an acquired taste, so let’s get practical. Microscopy is a primary tool of modern biology and medicine. In conjunction with structural, immunological, radioactive, or molecular markers, microscopy is used to distinguish exquisite differences between cells and helps define how they interact with each other. In particular, microscopy is crucial to the study of tissue. Tissue is characterized by assemblies of cells working together to perform a particular function. In today’s session, you will investigate epithelial and connective tissues to make this point.

What about the limits of reductionism? Reductionism is in a constant, dynamic tension, opposed by the fact that at each level of organization properties emerge that simply cannot be explained by studying individual parts. If somebody put all the parts of an automobile in front of you, for example, you could not predict what the top speed of the car would be even if you were an expert. “Speed” is a property of all the parts working together, an “emergent property” of assembled auto parts. (Look up “emergent properties” in your basic biology text, Campbell, for a brief discussion of this point.)

In tissue biology and pathology, for example, it becomes quickly apparent that a strictly reductionist approach doesn’t always work. Breaking tissue up into its constituent cells and studying them in isolation of their normal neighbors destroys vital information about how cells normally interact with each other. One must go back to the original level of organization to seek answers. The microscope enables us to study cell-cell interactions by inferring function from cell shape and surfaces in tissues such as the lung, where molecules, e.g., oxygen, move into the body and the intestine where nutrients in solution are brought into the body.

There are five major tissue types in animals: Blood, muscle, nerve, epithelial, and connective. We will focus on the last two. Typically, when we think of tissue, we think of organs such as the liver and kidney, or structures such as the stomach. In a single laboratory session, it is impractical to survey the many cell types that comprise various tissues. Instead, the goal of this section is to provide you with an understanding of the structural relationship between two major tissues: Epithelium and connective tissues that form organs and tubes. This knowledge will help organize your thinking should you study tissue at a later time.
Epithelial and stromal "compartments" separated by a basement membrane.

**Epithelial cells** form sheets that cover surfaces and line cavities and tubes. They function as the interface between inside and outside. The lining of the gut consists of a sheet of epithelial cells, for example.

**Fibrous connective tissue.** Epithelial structure is always supported underneath by a type of connective tissue, fibrous connective tissue. The name derives from the predominant cell type, the *fibroblast* and the characteristic fibers made of *collagen*, that holds the tissue together (Fig. 1). You are already familiar with connective tissue in the form of tendons, bone, and cartilage; loose fibrous connective tissue, the kind found beneath most organ epithelia, is less dramatic than a tendon or a bone, but is more plentiful, and is crucial for keeping body parts together. Another common term for loose fibrous connective tissue is "stroma."

**Basement membranes separate compartments.** The third important structure to keep in mind forms a permeable boundary between epithelium and stroma, and is called a "basement membrane" (or basal lamina) (Fig. 1). The basement membrane is secreted by the epithelium and to some extent fibroblasts. It is non-cellular and comprised of collagens in the adult, and mucopolysaccharides in the embryo. It plays a very important role during development, providing a foundation for epithelial cells to move and anchor. Basement membranes are important in tumor metastasis because tumor cells must breach this barrier in order to spread.

**Complex epithelial organs.** When sheets of epithelia pocket and fold, glands and other complicated structures, such as the lung, can be formed. Figure 2 illustrates the appearance of a branched tubular epithelium when viewed in a thin section. It is difficult for a beginning microscopist to visualize the complete 3-dimensional structure from observing only a thin slice of it.
Figure 2. The appearance of a branched tubular epithelium when viewed in a thin section. The gray background represents stroma.
III Setting up the Microscope

Place a slide on stage and attempt to focus on the specimen. Begin with the lowest power objective (4x), crank it down and focus upwards. Never make gross movements of the scope downward. This can break slides and damage the objective. Is the image too dark? Make sure the substage condenser is raised so that its lens is close to the bottom of the slide.

Be sure to set the interpupillary distance and focus each eye independently as you learned with your stereo microscopes earlier.

IV. Assignment

Using prepared slides of skin, intestine/stomach, kidney, and lung, investigate epithelial/stromal relationships. Study slides in the above order since this will take you from relatively simple to rather complex arrangements. Additional tissue may also be available if you master these.

**PREPARE DETAILED LABELED DRAWINGS OF TWO TISSUES OF YOUR CHOICE.** Each drawing should be in color, on a separate page and each should be at least 4” X 4”. Use the remainder of the page to describe what you have drawn. You may wish to do several drawings of the same tissue at different magnifications.

Be sure that the drawing and descriptions represent the epithelium/basal lamina/stroma motif in the context of the particular specimen.

**Skin.** Compare hair follicles and sweat glands. These are 2 simple variations on the “pocketing” technique for forming organs (Fig. 2).

**Intestine.** Are there plentiful white blood cells in the stroma underlying the villi?

**Lung.** Illustrate a bronchiole with attendant blood vessels. Distinguish between vein and artery. Is there stroma beneath the thin alveolar epithelium? Are there blood vessels (capillaries)? How might you identify them?

**Kidney** Find the basal lamina that responsible for retention of the serum proteins in glomerular filtration.
V. CARE OF MICROSCOPES

Mechanical damage to microscopes is far more common than damage to the lenses or other optical components. Mechanical damage occurs when the microscope is dropped or shoved off the bench onto the floor or when someone applies brute force when trying to move a mechanical part that is either not intended to move or whose lubrication has become stiff with age.

Rules that follow from this are:

• Take care not to drop the scope when taking it out of the cabinet, putting it away or when carrying it around the lab. If you will not be using the scope again during the lab period, put the scope in the storage cabinet rather than letting it sit on the bench top where it could be accidentally knocked off.

• Never apply excessive force to move any part of the microscope. Ask for help if something doesn't move freely and smoothly when you think it should.

Cleaning the Optical Components:

• Clean the lenses only with the lens tissue and lens cleaning fluid provided.

• Don't clean a lens unless you have reason to believe it is dirty. Knowing when a lens is dirty is an art that comes with experience. The oil immersion objective should be cleaned at the end of each lab period (if it has been used). The eyepiece lenses are frequently soiled by dust or nose prints.

• By far the most common problem experienced by the novice microscopist is accidentally getting immersion oil onto the surface of the 40X objective. Inability to produce a clear, crisp image with the 40X objective is the symptom of this problem. The problem can be avoided by NEVER changing objective magnification from 100X to 40X without FIRST removing immersion oil from the coverslip.

• Always keep the microscope covered when it is not in use.